Biological Characteristics and Specific Divergence of Fishes in Genus *Lateolabrax* (Perciformes: Percichthyidae)

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Contents

1 Introduction

Sea bass of the genus ***Lateolabrax*** (Perciformes: Percichthyidae) are popular and important commercial fishes in east Asian countries. Recently in Japan, they have also become very popular as targets by lure fishing. In particular, ***Lateolabrax japonicus*** is important for fisheries and artificial propagation is vigorously studied in Japan (Hayashida et al., 1984; Kumagai et al., 1984; Atsumi et al., 1994; Kimura, 1996; Makino, 2002), Korea (Myoung et al., 1997; Kim et al., 1998; Lee et al., 1998; Park et al., 1998; Kim et al., 2002), Taiwan (Perng et al., 1980; Tang et al., 1980; Perng et al., 1981; Perng and Liu, 1982; Perng and Liu, 1983; Tang, 1985; Huang et al. 1987; Huang and Tang, 1988; Huang and Tang, 1990; Liao, 1993; Kuo, 1993; Zheng et al., 1993) and China (Ruan, 2001; Zhang et al., 2001a, 2001b; Gao et al., 1998).

The genus ***Lateolabrax*** belongs to the family Percichthyidae, suborder Percoidei, order Perciformes, class Osteichthyes. The genus comprises a number of species and they have restricted distribution around east Asian coasts. As for the family, it has been considered to belong to Percichthyidae, however presently the taxonomic classification may be uncertain and there is even an opinion that it should belong to the family Moronidae (Waldman, 1986). The suborder Percoidei is considered not to be monophyletic (Nelson, 1994), and the family Percichthyidae contains many genera, obviously suggesting that it is a polyphyletic taxon. The genus ***Lateolabrax***, in particular, is a minor group in the Percichthyidae because it is characterized as having a small number of vertebrae and so on.

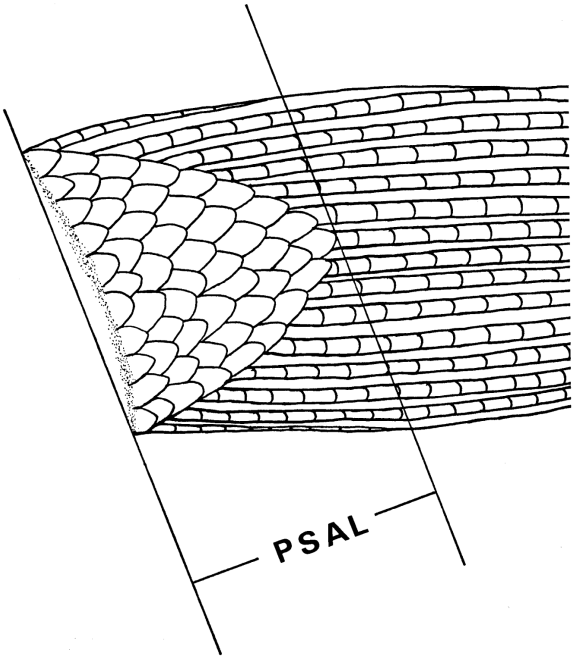

Fig. 1. Definition of pectoral scaly area length (PSAL).

The genus ***Lateolabrax*** was established by Bleeker (1854-57) for a single species, ***Lateolabrax japonicus*** (Cuvier). Thereafter, Katayama (1957) described a second species, ***Lateolabrax latus***. More recently, the author detected one more species in the genus by morphological and genetic examinations. Concerning this, it was also revealed that there are some biologically peculiar populations in ***L. japonicus*** such as the Ariake and Yatsushiro forms. Including these matters, this paper presents the biological characteristics and the specific diver-

gence of the new species in the genus ***Lateolabrax***.

2 Common methods

The fish specimens used in this study were examined morphologically and genetically. For the latter, isozymes were mainly used as genetic markers. Here, the common methods and techniques of morphological and genetic examinations of the isozymes will be noted.

Morphological analysis

The morphological examinations were made for fixed specimens with 10% formalin. The methods of morphological measurements and counts followed Hubbs and Lagler (1970). Vertebrae were counted using radiographs. For individuals which have some black dots on the body, the total of recognizable black dots on the left side of the body were counted, including those on the mid-dorsal aspect of

Table 1. Enzymes, protein and tissues examined

Enzyme or protein name	Enzyme number	Locus	Tissue
Aspartate aminotransferase	2.6.1.1	***AAT-1****	Liver
		AAT-2*	Liver
Alcohol dehydrogenase	1.1.1.1	***ADH****	Liver
Adenylate kinase	2.7.4.3	***AK****	Liver
Creatine kinase	2.7.3.2	***CK****	Muscle
Esterase	3.1.1.-	***EST-1****	Liver
		EST-2*	Liver
Fructose biphosphate aldolase	4.11.2.3	***FBALD-1****	Muscle
		FBALD-2*	Muscle
Glycerol-3-phosphate dehydrogenase	1.1.1.8	***G3PDH-1****	Liver
		G3PDH-2*	Muscle
Glucose-6-phosphate isomerase	5.3.1.9	***GPI-1****	Muscle
		GPI-2*	Muscle
L-Iditol dehydrogenase	1.1.1.14	***IDDH****	Liver
Isocitrate dehydrogenase ($NADP^+$)	1.1.1.42	***IDHP-1****	Liver
		IDHP-2*	Muscle
Lactate dehydrogenase	1.1.1.27	***LDH-1****	Muscle
		LDH-2*	Muscle
Malate dehydrogenase	1.1.1.37	***MDH-1****	Liver
		MDH-2*	Liver
Malic enzyme ($NADP^+$)	1.1.1.40	***MEP****	Muscle
Mannose-6-phosphate isomerase	5.3.1.8	***MPI-1****	Liver
		MPI-2*	Liver
Phosphogluconate dehydrogenase	1.1.1.44	***PGDH****	Liver
Phosphoglucomutase	5.4.2.2	***PGM****	Muscle
General protein		***PROT-1****	Muscle
		PROT-2*	Muscle
Superoxide dismutase	1.15.1.1	***SOD-1****	Liver
		SOD-2*	Liver

the caudal peduncle. Because squamation on the pectoral base may be significant, a new character, pectoral scaly area length (PSAL), was defined (Fig. 1).

Isozyme analysis

Isozymes were detected by horizontal starch-gel electrophoresis. Before the electrophoresis, the raw specimens were preserved in a refrigerator at -15－-80℃. The experimental methods were based mainly on Taniguchi and Okada (1980), using citric acid-aminopropylmorpholine buffer (pH 6.0) in each case.

Enzymes and a protein detected by electrophoresis were shown in Table 1. Seventeen enzymes and one non-enzymic protein were available for genetic markers and twenty nine loci were presumed in total (Table 1). However, some of those loci were omitted to examine in some earlier experiments.

Gene nomenclature followed Shaklee et al. (1990), the alleles being symbolized as relative mobility percentages compared with the most dominant alleles identified.

3 Morphological and genetic differences between Japanese and Chinese forms of *Lateolabrax* "*japonicus*"

During recent aquaculture operations in Japan, inexpensive and easily obtained seeds of various fish species from foreign countries have been vigorously introduced. In particular, sea bass ***Lateolabrax japonicus*** have been abundantly imported from China, Korea and Taiwan, mainly to western Japan (Matsuoka, 1993). Importation from China began in 1990, when about 1 million young sea bass were imported through a joint venture in Hong Kong, the quantity thereafter increasing

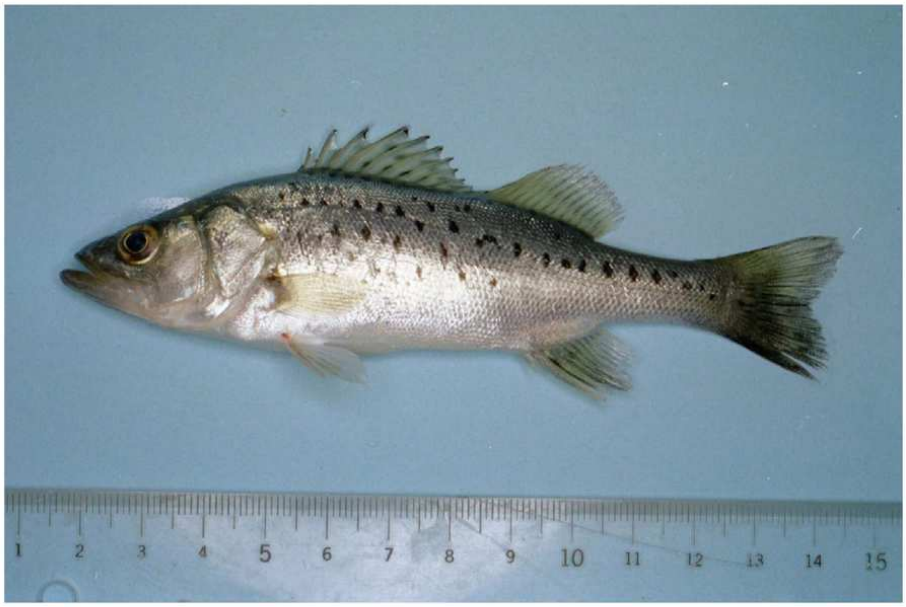

Fig. 2. General aspects of ***Lateolabrax*** sp. (KPFES 90003, 124.8 mm TL).

with each succeeding importation and reaching 4 million in 1992.

Most imported sea bass are characterized by many clear black dots on the lateral body region (Fig. 2). It is common knowledge in the sea bass nurseries that such dots do not disappear with growth, as in sea bass from Japanese waters. In particular, the sea bass from China, which constitute the bulk of imported sea bass, display this feature prominently.

Sea bass with many clear black dots have been recently caught by anglers and fishermen in fishing grounds in western Japan. These fish are considered to be Chinese sea bass, since such are known to have escaped from some nurseries.

Consequently, the effects of the existence of free-living Chinese sea bass in Japanese waters is of concern, necessitating examination of their biological features. For this reason, the study of this section was carried out to clarify morphological and genetic differences between sea bass from Japan and China.

Materials and methods

Data from specimens used for morphological analyses are shown in Table 2. The sea bass from Japan (hereafter called Japanese form) were collected from a branch of the Yoshino River, Tokushima City, and from southern Harima Sea, Seto Inland Sea. The sea bass from China (hereafter called Chinese form) were sampled from aquaculture seeds, which were transported from China via Hong Kong to Kagawa Prefecture. Although the exact collection locality is uncertain, information

Table 2. Collecting and treating data of examined specimens

	Examination for morphology	
Population	Japanese	Chinese
Date of collection	30 Oct. 1988／11 Dec.1990	17 June 1990
Locality	Tokushima ／ Harima Sea	Chinese coast
Method of sampling	Angling ／ Trawl net	Transported from Hong Kong
Number of individuals	20 ／ 45	62
Range of size (TL,mm)	149.8－280.0 ／247.0－493.0	90.4－143.0
Average size (TL,mm)	198.7 ／ 440.6	116.2
Preservation	70% ethanol ／ 10% formalin	10% formalin
Catalog No.	TKPM-P352 ／ KPFES 90007	KPFES 90001-6, TKPM-P1655
	Examination for genetics	
Population	Japanese	Chinese
Date of collection	11 Dec. 1990／17 Dec. 1991	17 June 1990
Locality	Harima Sea ／ Harima Sea	Chinese coast
Method of sampling	Trawl net ／ Trawl net	Transported from Hong Kong
Number of individuals	45 ／ 27	58
Range of size (TL,mm)	247.0－493.0 ／ Unmesured	90.4－143.0
Average size (TL,mm)	440.6 ／ Unmesured	116.3
Preservation	Frozen in -15℃／Frozen in -80℃	Frozen in -80℃
Catalog No.	KPFES 90007 ／ unpreserved	TKPM-P1655

from buyers suggested that the imported fish were caught from coastal waters of the Bohai Sea or Yellow Sea. The specimens examined at this section have been deposited in the Tokushima Prefectural Museum (TKPM) and the Kagawa Prefectural Fisheries Experimental Station (KPFES).

Data from specimens used for genetic analyses are also shown in Table 2. Japanese forms were collected from the southern Harima Sea by trawl. Chinese forms comprised mostly those used for the morphological analyses. For the specimens, isozymes were examined as genetic characters.

The obtained specimens were morphologically and genetically examined with the common methods noted at section 2.

Results of morphological characters

Proportional measurements are shown in Table 3. Some proportional differences between the two forms were recognized, notably the orbital diameter (OD), which was greater in the Chinese form (Table 3, Fig. 3). In addition, the PSAL in the Chinese form was distinctly shorter than in the Japanese form (Table 3, Fig. 4). Histograms of PSAL frequencies in the two forms are shown in Fig. 5. Both re-

Table 3. Proportions of length-measured characters of Japanese and Chinese forms of ***Lateolarax***

Population	Japanese		Chinese	
	Average	Range	Average	Range
Total length[1]	121.69	117.25 — 128.00	119.17	114.61 — 125.45
Fork length[1]	115.11	111.14 — 121.09	114.00	111.11 — 118.33
Pre-anus length[1]	66.04	62.35 — 69.31	66.76	63.70 — 69.94
Body depth[1]	24.24	20.35 — 29.12	26.28	24.34 — 28.76
Body width[1]	13.38	10.42 — 16.43	13.46	12.11 — 18.78
Caudal peduncle depth[1]	9.28	7.77 — 11.04	10.16	9.35 — 10.89
Caudal peduncle length[1]	21.61	18.70 — 24.64	22.09	20.21 — 25.70
Pre-dorsal length[1]	35.24	33.33 — 38.31	35.00	31.99 — 39.29
First dorsal fin length[1]	14.10	11.99 — 17.04	12.62	10.24 — 14.73
Second dorsal fin length[1]	11.82	9.19 — 13.89	12.57	10.58 — 14.91
Anal fin length[1]	12.49	10.05 — 14.90	14.33	11.84 — 16.67
Pectoral fin length[1]	17.05	14.84 — 19.51	16.08	13.67 — 17.81
Pelvic fin length[1]	17.57	15.05 — 19.58	18.33	16.30 — 21.05
Head length[1]	31.98	29.16 — 35.46	32.56	30.67 — 33.65
Snout length[2]	26.25	21.95 — 29.64	24.06	19.43 — 26.81
Orbital diameter[2]	17.70	14.34 — 22.71	24.83	21.31 — 30.49
Interorbital width[2]	21.20	17.38 — 24.48	21.64	18.18 — 24.14
Sub-orbital width[2]	11.26	6.64 — 14.45	10.72	6.96 — 15.91
Upper jaw length[2]	42.36	38.36 — 46.51	44.21	40.63 — 50.00
Lower jaw length[3]	46.43	42.44 — 49.51	46.82	43.71 — 51.70
Pectoral scaly area length[3]	26.73	18.69 — 36.51	19.43	14.79 — 23.78

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

[3] Percentage of pectoral fin length

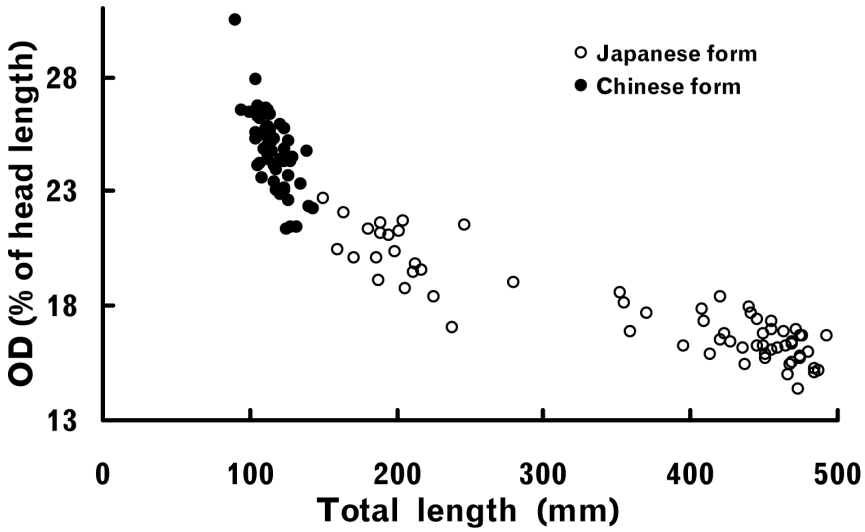

Fig. 3. Relationship between total length and percentage of orbital diameter (OD) of head length.

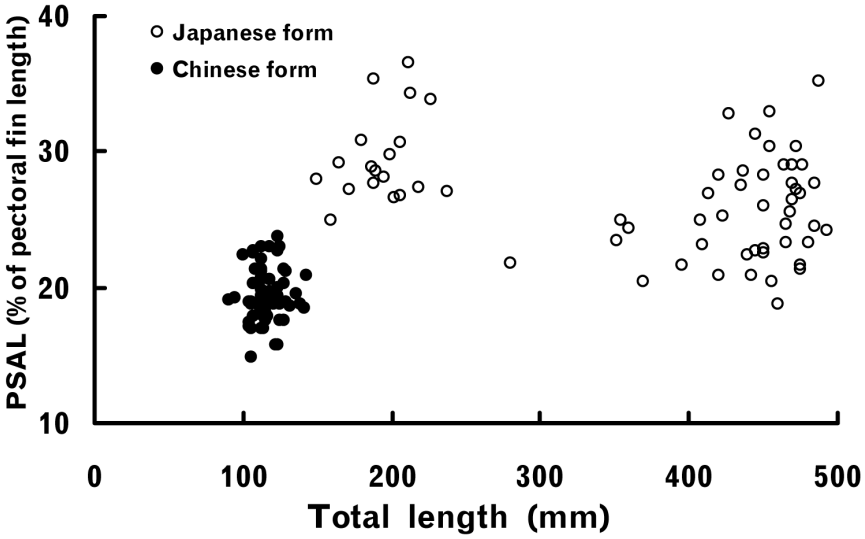

Fig. 4. Relationship between total length and percentage of pectoral scaly area length (PSAL) of pectoral fin length.

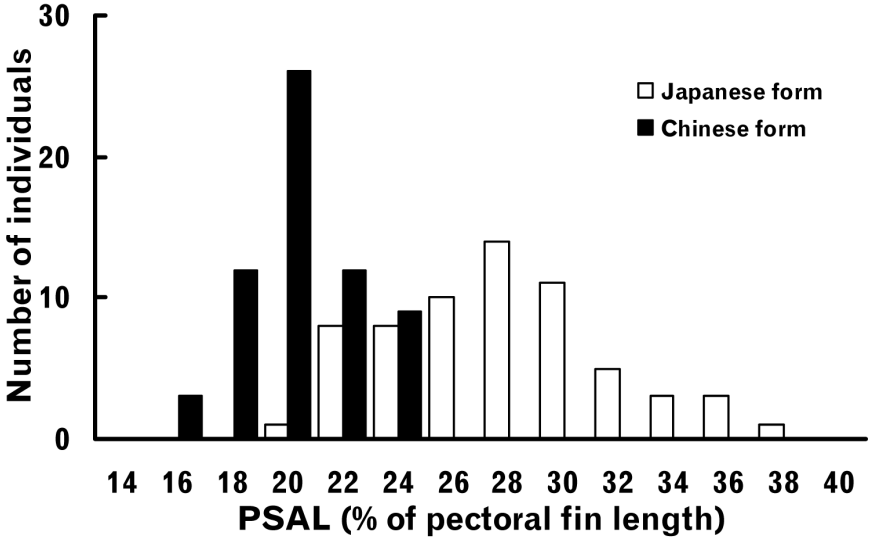

Fig. 5. Frequency distribution of pectoral scaly area rate (PSAL).

Table 4. Meristic counts of Japanese and Chinese forms of ***Lateolabrax***

Population	Japanese		Chinese	
	Average	Range	Average	Range
Dorsal fin spines	12.85	12 — 14	12.95	12 — 14
Dorsal fin soft rays	12.78	12 — 14	13.06	12 — 14
Anal fin spines	3.00	3 — 3	2.98	2 — 3
Anal fin soft rays	7.73	7 — 9	7.53	6 — 8
Pectoral fin soft rays	16.85	15 — 18	16.31	15 — 18
Pelvic fin spines	1.00	1 — 1	1.00	1 — 1
Pelvic fin soft rays	5.00	5 — 5	5.00	5 — 5
Pored scales on lateral line	83.08	76 — 92	72.85	66 — 82
Scales above lateral line	14.85	13 — 17	15.82	13 — 17
Scales below lateral line	19.37	17 — 22	19.18	16 — 22
Gill rakers (upper limb)	9.66	8 — 11	6.38	5 — 8
Gill rakers (lower limb)	17.52	16 — 20	14.70	13 — 17
Gill rakers (Total)	27.18	24 — 30	21.07	19 — 24
Vertebrae	35.89	35 — 37	34.95	34 — 36

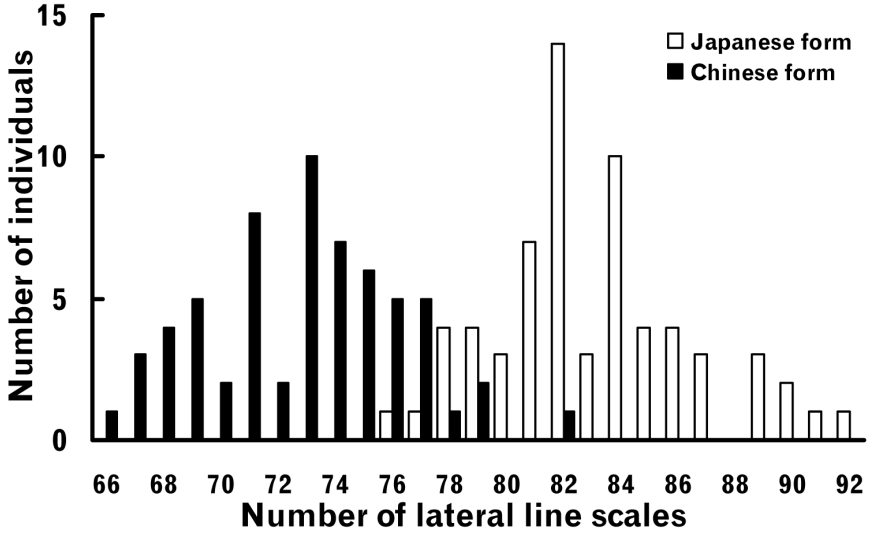

Fig. 6. Frequency distribution of lateral line scales (LLS).

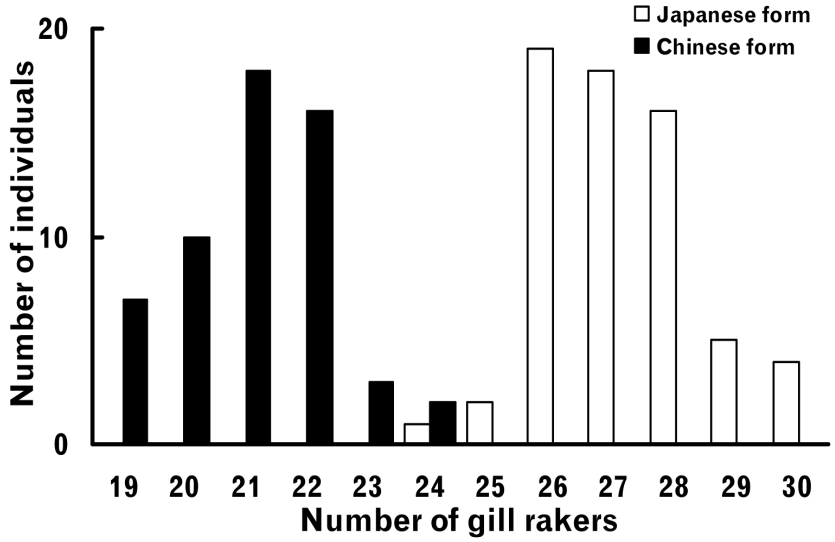

Fig. 7. Frequency distribution of total gill rakers (GR).

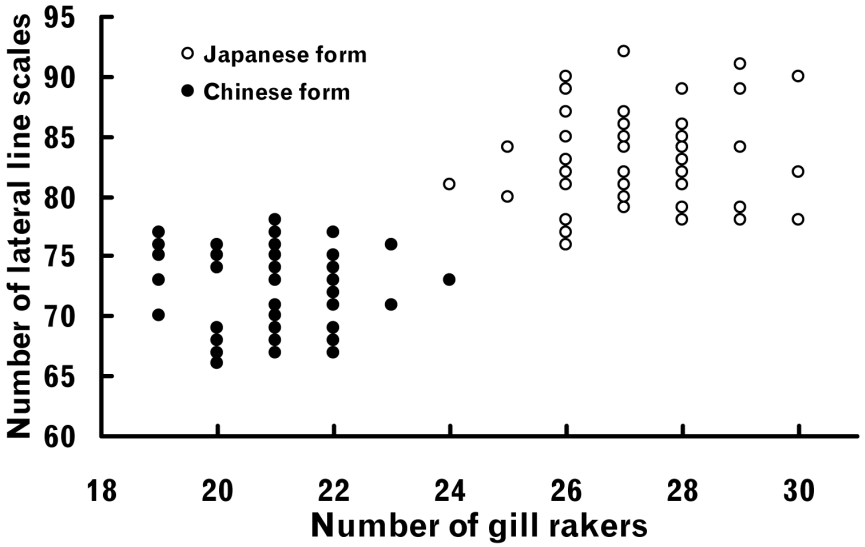

Fig. 8. Relationship between gill rakers (GR) and lateral line scales (LLS)

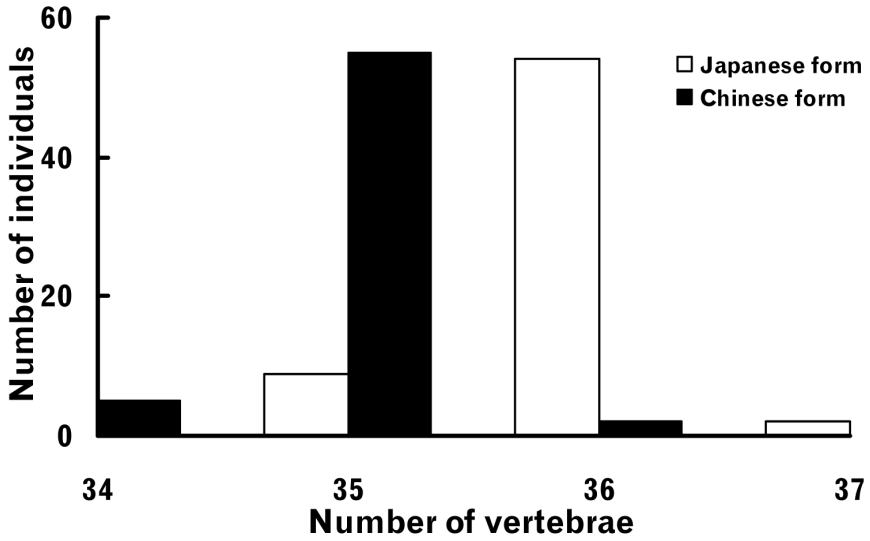

Fig. 9. Frequency distribution of vertebrae (VT).

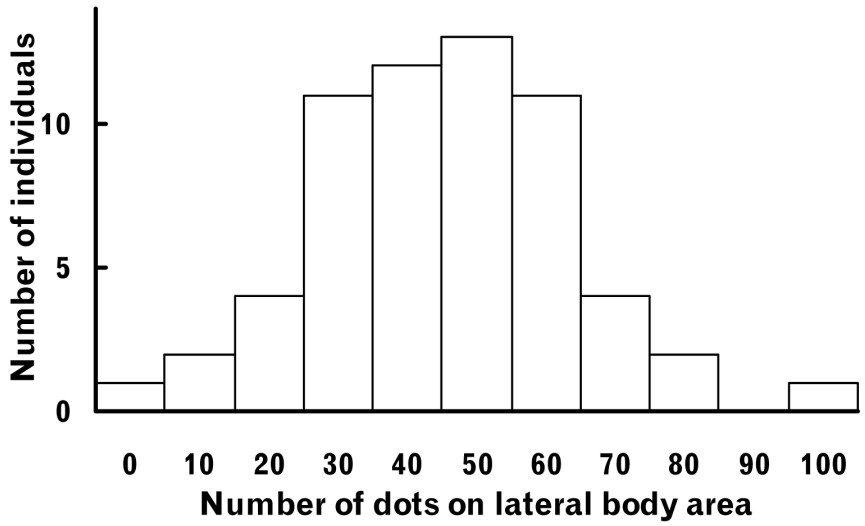

Fig. 10. Frequency distribution of dot numbers on lateral body area in Chinese form.

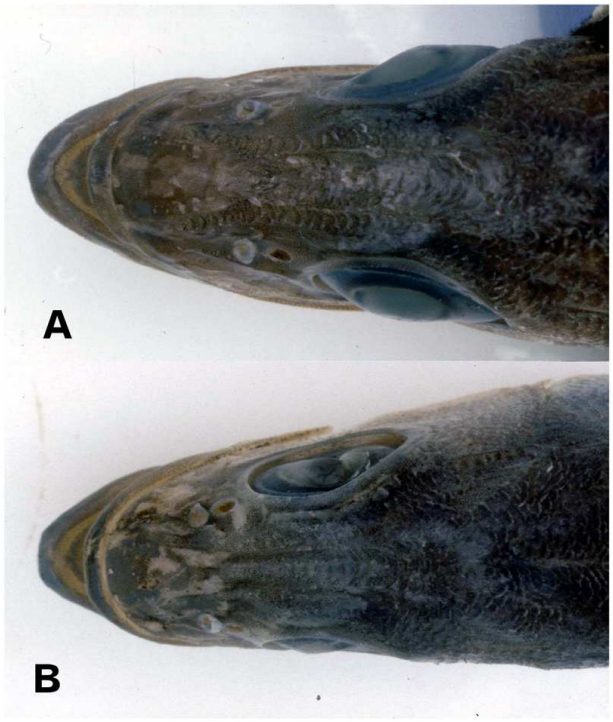

Fig. 11. Dorsal aspects of head. A: Japanese form (TKPM-P352, 149.8 mm TL). B: Chinese form (KPFES 90004, 112.5 mm TL).

sulted in normal distributions and indicated clear differences in shape and mode.

Average values and ranges of the meristic counts, except number of lateral dots, are shown in Table 4, the average values of the meristics differing between the two forms. The differences in pored lateral line scales (LLS) and total gill rakers (GR) were obvious, the average values of these characters differing by nearly 10 and 6, respectively, between the two forms. Although LLS and GR ranges overlapped (Figs. 6, 7), the combination of GR and LLS counts separated the two forms completely (Fig. 8). Further, difference in vertebral counts (VT) is also significant (Fig. 9).

A histogram of the number of lateral dots on the Chinese form indicated a normal distribution, ranging from 0 to nearly 100, with a mode of 40−50 (Fig. 10). This character was a typical feature of the Chinese form, few individuals lacking dots.

The squamation on the dorsal surface of the head differed in the two forms (Fig. 11). In the Japanese form, two scale rows extended from the interorbital region, reaching anteriorly beyond the nostrils (Fig. 11 A). In the Chinese form, however, the scale rows were restricted to the interorbital region, not extending beyond the nostrils (Fig. 11 B). This difference in squamation provides a means of identifying the two forms in the fingerling and juvenile stages.

Key characters for identification of ***L. japonicus*** and ***L. latus*** (Katayama, 1957, 1960a, 1960b) were also examined in the two forms. Dorsal fin rays numbered 12−14 in both (Table 4), with scale counts below the lateral line in each almost exactly corresponding with those of ***L. japonicus*** given by Katayama (1957, 1960a, 1960b). No individuals of either form had scales on the ventral surface of the lower jaw.

Results of genetic characters

Here, 20 isozymic loci detected by electrophoresis were used as the genetic

Table 5. Allelic frequencies of Japanese and Chinese forms of ***Lateolabrax***

Locus	Allele	Frequency	
Population		Japanese	Chinese
*AAT-1**	***100**	0.792	0.981
	***85**	0.208	0.019
*AAT-2**	****-100**	1.000	1.000
*ADH**	****-50**	0.011	0.000
	****-100**	0.659	1.000
	****-150**	0.330	0.000
*CK**	***100**	1.000	1.000
*G3PDH-1**	***100**	1.000	1.000
*G3PDH-2**	***100**	0.986	1.000
	****-200**	0.014	0.000
*GPI-1**	***130**	0.000	0.060
	***110**	0.100	0.922
	***100**	0.900	0.018
*GPI-2**	****-100**	0.937	0.940
	****-250**	0.063	0.060
*IDDH**	***165**	0.037	0.200
	***100**	0.963	0.800
*IDHP-1**	***100**	0.912	0.588
	***70**	0.088	0.412
*LDH-2**	***100**	0.097	0.966
	****-100**	0.903	0.034
*MDH-1**	***100**	0.986	1.000
	***70**	0.014	0.000
*MDH-2**	***100**	1.000	1.000
*MEP**	***150**	0.079	0.050
	***100**	0.886	0.900
	***50**	0.035	0.050
*MPI-1**	***125**	0.306	0.363
	***100**	0.694	0.638
*MPI-2**	***100**	1.000	0.987
	***75**	0.000	0.013
*PGDH**	***120**	0.000	0.011
	***100**	1.000	0.989
*PGM**	***115**	0.021	0.060
	***100**	0.465	0.500
	***75**	0.507	0.431
	***55**	0.007	0.009
*PROT-1**	***170**	0.000	1.000
	***100**	1.000	0.000
*SOD-1**	***100**	1.000	0.964
	***20**	0.000	0.036

markers (Table 5). Allelic frequencies of the loci with χ^2 heterogeneities between both forms are shown in Table 5, with some electrophoretograms of significant isozymes illustrated in Fig. 12.

Initially, the fitness of the allelic frequencies in polymorphic loci, according to Hardy-Weinberg equilibrium, was examined by chi-square test. Because no χ^2 values were significant at the 5% level, it was considered that the two sea bass forms originated from simple Mendelian populations.

Regarding to the heterogeneities between the groups, chi-square tests indicated significant differences in many of the loci examined (Table 5). Some of these loci and alleles are described as follows.

Alcohol dehydrogenase (ADH): The ***ADH**** locus of the Japanese form had a major allele of ****-100***, and two minor ones of ****-50*** and ****-150***, whereas the locus in the Chinese form was monomorphic, being entirely occupied by the ****-100*** allele (Fig. 12; Table 5).

Glucose-6-phosphate isomerase (GPI): At the ***GPI-1**** locus of the Japanese form, a major allele (****100***) with a frequency of 0.900, and a minor one (****110***) were found, whereas the Chinese form had the ****110*** allele as the major allele, with a frequency of 0.922, and minor ones of ****100*** and ****130*** (Fig. 12; Table 5).

Lactate dehydrogenase (LDH): At the ***LDH-2**** locus of the Japanese form, the major allele of ****-100*** had a frequency of 0.903, with a minor allele of ****100***. In contrast, in the Chinese form, the major and minor alleles were essentially reversed, with the major allele being ****100*** (frequency 0.966) and the minor one ****-100*** (Fig. 12; Table 5).

General protein (PROT): The ***PROT-1**** locus of the Japanese form was occu-

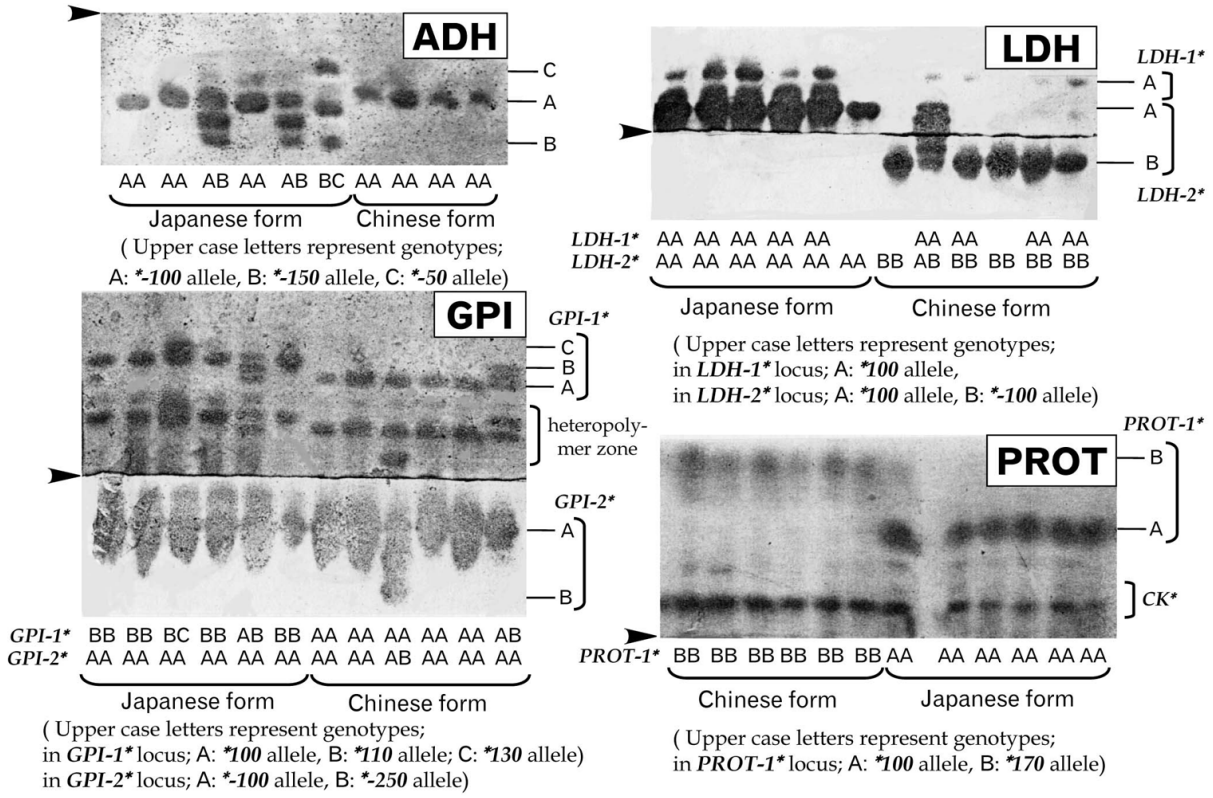

Fig. 12. Selected electrophoretograms of some loci in Japanese and Chinese forms.

pied by an allele of ***100**. That of the Chinese form was occupied by an allele of ***170**, the alleles having completely replaced each other (Fig. 12; Table 5).

Of the other isozymes, significant differences in allelic frequencies between the two forms were found in ***AAT-1***, ***IDDH***, ***IDHP-1*** and ***SOD-1*** (Table 5).

The genetic distance (D value) between the two forms, calculated from isozymic allele frequencies after Nei (1972) was 0.174, far above the D values of some Japanese sea bass populations examined by Tsuda (1989), and significant at the inter-specific level (Nei, 1990).

Discussion

The morphological and genetic differences indicated that the Chinese form is a distinct species from ***L. japonicus***. This is also supported by the absence of genetic exchange between the groups, there being complete allele replacement at the ***PROT-1*** locus. The Chinese form also differed from ***L. latus*** in the number of dorsal fin rays and scales below the lateral line, and scalation on the ventral side of the lower jaw.

For this reason, the Chinese form is here referred to as ***Lateolabrax*** sp. until formally described. ***Lateolabrax japonicus*** is supposedly widely distributed in coastal waters of the Japanese Archipelago, Korea, China and Taiwan (Katayama, 1965a; Miyadi et al. 1976). This study showed that a distinct species, formerly

confused with ***L. japonicus***, inhabits the Chinese coast.

Although Katayama (1960a, 1960b) pointed out that sea bass from Chinese waters typically possessed many black dots along the lateral body surface, he regarded it as an intraspecific variation of ***L. japonicus***. Consequently, his meristic and morphometric characters for ***L. japonicus*** included the two species. Thereafter, Yamada (1986) also included both species in his description of ***L. japonicus***.

According to some descriptions of so-called ***L. japonicus*** in China including Hon Kong, illustrations and meristics for LLS and GR, all point to ***Lateolabrax*** sp. (Zhu et al., 1963; Chang and Tang, 1968; Cheng and Zheng, 1987; Zheng, 1989; Liu and Qin, 1987; Pan et al., 1991). Recent studies on the morphology (Chen et al., 2001; Lou et al., 2002) and genetics (Lou et. al., 2003) between the Japanese and Chinese sea bass, which were carried out in China, indicated quite similar results to those in this section.

There is also some ecological differentiation between the two species. The general spawning season of ***L. japonicus*** in Japan is from December to March (Ochiai and Tanaka, 1986), whereas in the Bohai Sea, where the species is believed to be ***Lateolabrax*** sp., spawning is reported to occur from August to November (Liu and Qin, 1987; Deng et al., 1988; Jiang et al., 1988; Wan and Chen, 1988).

A difference in preference for fresh water is also apparent. Although ***L. japonicus*** is a marine species, it occasionally occurs in brackish or low salinity water in rivers (Miyazi et al., 1976). The Chinese species has a rather stronger tolerance of fresh water than ***L. japonicus***, being able to survive in completely fresh water (Zhu et al., 1963; Liu and Qin, 1987). In a river in southern China, it has been reported from areas more than 300Km from the mouth (Zheng, 1989).

In Taiwan, native sea bass, which are here thought to be conspecific with ***Lateolabrax*** sp. owing to their appearance, are cultivated in fresh-water ponds by acclimatization. They have been reported to grow as well in fresh-water conditions as in sea water, reaching maturity and spawning (Perng et al., 1980; Tang et al., 1980; Perng et al., 1981; Perng and Liu, 1982; Perng and Liu, 1983; Tang, 1985; Huang et al. 1987; Huang and Tang, 1988; Huang and Tang, 1990). ***Lateolabrax japonicus***, however, although euryhaline, has never been known to grow or spawn in fresh water.

A difference in growth rate of the two species has been noted. According to Ochiai and Tanaka (1986), who summarized growth data of ***L. japonicus*** from various localities in Japan, growth rates generally do not vary locally, lengths being around 20cm after the first year, 30cm after 2 years and 40cm after 3 years. ***Lateolabrax*** sp. has been reported to reach around 30cm after the first year, 40cm after 2 years and 50cm after 3 years (Wu et al., 1979; Liu and Qin, 1987). In artifi-

cial ponds in Taiwan, the difference is even greater, ***Lateolabrax*** sp. reaching around 20cm in the first 6 months (Huang and Tang, 1990) and almost 40cm after one year (Perng and Liu, 1982).

Satoh (1996) experimented on the growth rate of the Japanese and Chinese sea bass under the same condition in pen net cages, with the result that the Chinese one grew much more rapidly than the Japanese one. This suggests that the high growth rate of ***L***. sp. is not caused by environmental factors but by genetic factors.

In the Korean Peninsula, Korean information suggests that both species occur (Choi et al., 1990; Park et al., 1996; Kim and Jun, 1997; Park et al., 1999). Park et al. (1996) and Kim and Jun (1997) examined the isozymes and morphology of spotted and non-spotted forms of sea bass from Korea, respectively, both studies detecting considerable differences which correspond very well with those between ***L. japonicus*** and ***L***. sp. which have been revealed in this study. More recently in Korea, Kim et al. (2001) treated ***L. japonicus*** and ***L***. sp. separately, referring to ***L***. sp. as ***Lateolabrax maculatus*** (McClelland, 1884).

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4 Disturbance of Japanese native ecosystem by introduced species

Recently in some localities around western Japan, sea bass with many clear black dots on the lateral body region were caught by angling and so on, being introduced by mass media. These sea bass are called "hoshisuzuki" (Konishi, 1993; Weekly Sunday Fishing, 1993a; Weekly Sunday Fishing, 1993b; Yokoyama, 1994) or "hanten-suzuki" (Mainichi Newspaper, 1993; Lure Field, 1993).

The so-called "hoshisuzuki" (Fig. 13) is very similar to the Chinese sea bass ***Lateolabrax*** sp. in appearance, which is suggested to be a different species from the Japanese sea bass ***Lateolabrax japonicus*** in section 3. In various localities around western Japan, the Chinese sea bass is vigorously cultured in cages (Matsuoka, 1993) mainly in Ehime Prefecture. Since there is some information that many of the cultured sea bass escaped from the cages, the "hoshisuzuki" could be the cultured Chinese sea bass.

Therefore, the morphological characters and genetic features (isozymes) of the "hoshisuzuki" were compared with those of the Chinese and Japanese sea bass.

Materials and methods

The "hoshisuzuki" used as materials were 14 individuals in total, including 1 individual obtained offshore from Tsuda, eastern Kagawa Prefecture, 1 individual acquired offshore from Fukuda (Shodo Island, being located in northeastern Ka-

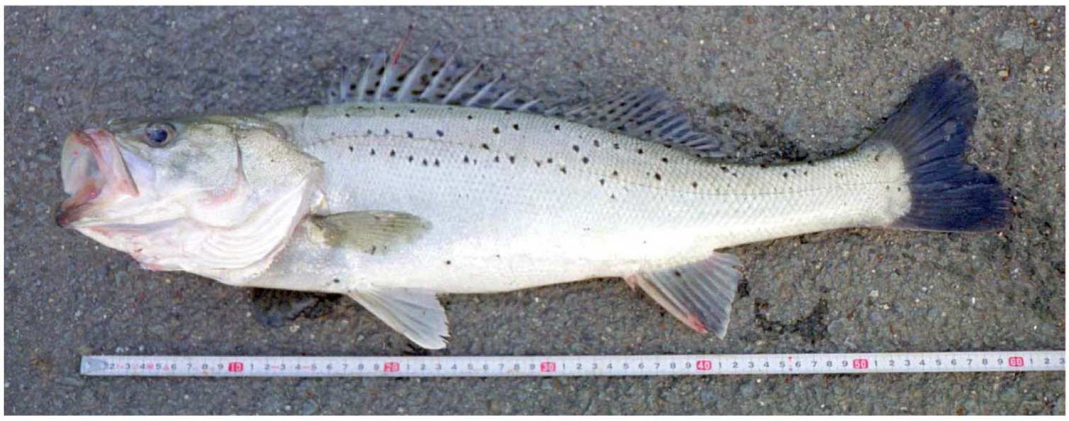

Fig. 13. General aspects of "hoshisuzuki" from Kunomura River, Uwajima, Japan (Individual No. U-3, 600mm TL).

Table 6. Sampling data for specimens of "hoshisuzuki" and non-spotted sea bass (genus ***Lateolabrax***)

Individual No.	Collecting date	Locality	Sampling method	Type	Total length (mm)	Catalog No.
T-1	7 May 1992	Tsuda off shore	Fixed shore net	"Hoshisuzuki"	186	
F-97	19 May 1993	Fukuda off shore	Fixed shore net	"Hoshisuzuki"	522	KPM-NI0009696
F-98	19 May 1993	Fukuda off shore	Fixed shore net	Non-spotted	395	KPM-NI0009697
F-99	19 May 1993	Fukuda off shore	Fixed shore net	Non-spotted	371	KPM-NI0009698
U-1	23 May 1993	Kunomura River	Lure fishing	Non-spotted	380	KPM-NI0009685
U-2	23 May 1993	Kunomura River	Lure fishing	"Hoshisuzuki"	420	KPM-NI0009690
U-3	23 May 1993	Kunomura River	Lure fishing	"Hoshisuzuki"	600	
U-4	23 May 1993	Kunomura River	Lure fishing	"Hoshisuzuki"	400	KPM-NI0009695
U-5	12 June 1993	Kunomura River	Lure fishing	"Hoshisuzuki"	451	KPM-NI0009686
U-6	12 June 1993	Kunomura River	Lure fishing	"Hoshisuzuki"	355	KPM-NI0009687
U-7	12 June 1993	Kunomura River	Lure fishing	"Hoshisuzuki"	338	KPM-NI0009688
U-8	12 June 1993	Kunomura River	Lure fishing	"Hoshisuzuki"	459	KPM-NI0009689
U-9	12 June 1993	Kunomura River	Lure fishing	Non-spotted	324	KPM-NI0009699
U-10	28 Nov. 1993	Kunomura River	Lure fishing	"Hoshisuzuki"	233	KPM-NI0009691
U-11	28 Nov. 1993	Kunomura River	Lure fishing	"Hoshisuzuki"	281	KPM-NI0009692
U-12	28 Nov. 1993	Kunomura River	Lure fishing	"Hoshisuzuki"	279	KPM-NI0009693
U-13	28 Nov. 1993	Kunomura River	Lure fishing	"Hoshisuzuki"	690	KPM-NI0009694
O-1	30 Nov. 1994	Osaka Bay	Gill net	"Hoshisuzuki"	532	

gawa Prefecture), 11 individuals taken from the estuary of Kunomura River in Uwajima City, Ehime Prefecture, and 1 individual obtained offshore from Izumi-sano, Osaka Prefecture. Their capture sites are shown in Fig. 14.

Also, some individuals of sea bass with no dots on the lateral body region which were presumed to be native ones (hereafter called non-spotted type) were caught at the same site at the same time, and those were also used for the materials. Detailed sampling data for specimens of "hoshisuzuki" and the non-spotted type are shown in Table 6.

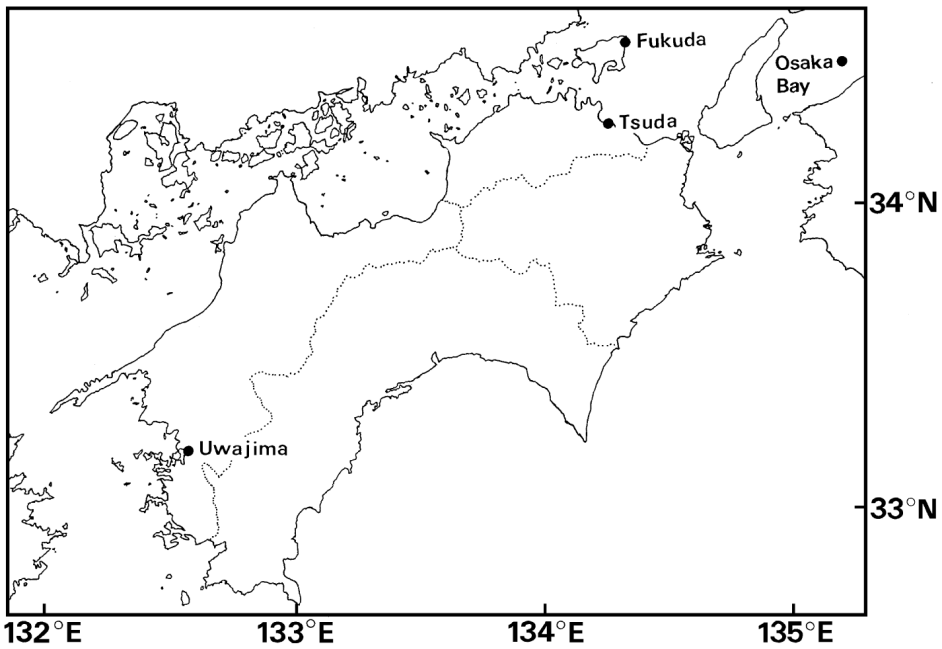

Fig. 14. Capture sites of ***Lateolabrax*** spp.

Table 7. Measurements and counts of "hoshisuzuki" and non-spotted sea bass (genus ***Lateolabrax***)

Individual No.	T-1	F-97	U-2	U-3	U-4	U-5	U-6	U-7	U-8	U-10	U-11	U-12	U-13	O-1	F-98	F-99	U-1	U-9
Type[1]	H	H	H	H	H	H	H	H	H	H	H	H	H	H	N	N	N	N
Standard length (mm)	152.0	440.0				364.0	303.0	283.7	375.0	196.0	230.0	229.4	570.0	435.0	336.0	317.0		268.6
Fork length[2]	114.47	114.32				118.96	112.87	114.56	117.60	113.57	112.39	115.43	112.28	113.10	114.58	113.56		113.18
Pre-anus length[2]	66.05	64.23				62.97	62.77	67.04	64.16	65.92	67.39	69.14	71.93	63.13	63.93	66.81		65.38
Body depth[3]	26.32	26.75				31.57	27.39	26.47	30.13	26.63	23.83	26.59	23.23	23.72	22.62	24.16		26.55
Body width[2]	14.08	13.36				15.93	16.50	15.65	15.31	13.57	14.39	14.73	14.04	13.93	13.81	14.67		15.26
Caudal peduncle depth[2]	9.74	9.73				11.07	10.63	10.65	10.51	10.31	10.43	10.03	9.02	9.56	9.64	9.56		9.98
Caudal peduncle length[2]	22.57	25.30				21.24	24.46	21.85	21.92	23.16	22.35	21.27	23.51	23.54	22.74	23.03		22.19
Pre-dorsal length[2]	33.16	32.36				36.62	34.95	32.64	35.15	34.18	32.70	35.18	32.25	36.74	32.74	33.66		35.00
First dorsal fin length[2]	12.63	12.68				15.08	13.66	13.04	15.23	12.86	14.17	15.17	12.42	11.36	12.08	13.19		13.81
Second dorsal fin length[2]	11.97	11.34				12.86	11.55	9.16	9.17	11.02	13.22	13.78	11.44	11.61	9.97	7.95		12.62
Anal fin length[2]	13.36	11.86				13.46	12.05	11.28	11.39	13.27	12.48	14.60	11.93	12.76	10.42	10.03		12.06
Pectoral fin length[2]	17.17	15.14				17.86	16.14	16.14	17.65	15.92	16.70	18.40	16.39	14.99	16.79	15.46		16.72
Pelvic fin length[2]	17.37	15.75				20.16	16.83	17.06	17.49	17.04	16.96	17.44	15.67	17.36	16.40	15.27		17.20
Head length[2]	31.78	29.66				34.07	32.74	31.02	33.60	32.19	32.30	34.26	31.65	30.44	29.35	28.71		30.86
Snout length[3]	22.36	23.37	26.12	24.65	24.01	23.23	23.69	22.73	22.62	21.87	18.98	22.14	25.39	22.36	29.21	29.23	28.51	27.38
Orbital diameter[3]	22.77	13.41	13.99	14.48	14.82	15.00	14.92	16.48	14.60	20.92	18.17	15.78	12.36	14.35	16.13	16.04	15.68	16.89
Interorbital width[3]	20.70	21.00	21.27	21.31	19.27	19.19	19.25	20.45	19.05	18.23	16.96	17.30	17.41	20.69	22.31	22.20	22.91	22.56
Suborbital width[3]	8.90	14.56	13.90	15.04	14.53	15.32	14.31	13.64	13.97	11.41	11.98	12.34	12.08	13.29	10.24	12.64	9.88	11.82
Upper jaw length[3]	43.06	40.69	40.30	41.78	40.12	41.45	41.94	42.27	40.87	43.74	43.07	43.89	41.57	40.71	42.39	45.05	42.26	42.82
Lower jaw length[3]	45.76	42.38	46.27	44.71	44.27	43.79	45.16	43.30	44.68	47.39	46.30	48.35	42.85	43.20	45.84	48.79	44.81	46.80
Pectoral scaly area length[4]	21.07	19.52				23.23	27.20	28.38	28.85	26.92	41.15	30.33	31.48	24.23	20.74	21.22		31.18
Dorsal fin spines	13	13				13	13	12	13	12	13	13	13	13	13	12		13
Dorsal fin soft rays	12	12				13	13	13	12	13	12	12	12	12	13	13		13
Anal fin spines	3	3				3	3	3	3	3	3	3	3	3	3	3		3
Anal fin soft rays	6	7				7	7	7	7	8	7	7	7	7	8	8		7
Pectoral fin soft rays	17	16				17	16	16	16	17	16	17	16	17	18	18		17
Pelvic fin spines	1	1				1	1	1	1	1	1	1	1	1	1	1		1
Pelvic fin soft rays	5	5				5	5	5	5	5	5	5	5	5	5	5		5
Pored scales on lateral line	83	72				76	73	72	74	77	74	78	78	80	77	83		82
Scales above lateral line	16	17				14	17	16	14	18	17	17	14	17	15	17		16
Scales below lateral line	22	19				21	20	19	20	22	20	20	19	21	19	17		19
Gill rakers (upper limb)	6	9	7	6	7	8	4	6	7	6	8	7	6	8	11	9	9	8
Gill rakers (lower limb)	17	16	16	16	15	16	15	16	15	16	16	16	15	16	16	17	17	17
Gill rakers (total)	23	25	23	22	22	24	19	22	22	22	24	23	21	24	27	26	26	25
Vertebrae	35	35				35	35	35	35	35	35	35	34	35	36	36		36
Dots on body	33	67				41	20	31	66	44	43	49	124	31	0	0		0

[1] H: "Hoshisuzuki"; N: Non-spotted sea bass [2] Percentage of standard length [3] Percentage of head length [4] Percentage of pectoral fin length

The obtained specimens were morphologically and genetically examined by the common methods noted in section 2. For number U1-U4 individuals, as only the head and a part of the internal organs were preserved, the characters in the head and the isozymes were examined. Most of the specimens examined in this section have been deposited in the Kanagawa Prefectural Museum of Natural History (KPM) (Table 6).

Results of morphological characters

The measurements and counts of "hoshisuzuki" and the non-spotted type examined are shown in Table 7. In length-measured characters, many individuals of "hoshisuzuki" had relatively larger body depth, caudal peduncle depth, head length and orbital diameter than those of the non-spotted type, corresponding well with the relationship between ***L. japonicus*** and ***L.*** sp., as reported in section 3. But the pectoral scaly area length (PSAL), which considerably differed from each other in ***L. japonicus*** and ***L.*** sp. (Fig. 5), did not show any difference between "hoshisuzuki" and the non-spotted type (Table 7)—both were similar to the values of ***L. japonicus***.

In meristic characters, it was revealed that ***L. japonicus*** and ***L.*** sp. differed from each other in pored lateral line scales (LLS), gill rakers (GR) and vertebrae (VT) (Figs. 6, 7, 9). The values of these characters of "hoshisuzuki" and the non-spotted type corresponded well with the relationship between the two former species (Table 7).

Figures 15—17 show the frequency distributions of LLS, GR and VT, respectively, in "hoshisuzuki", together with data for ***L. japonicus*** and ***L.*** sp. The histograms

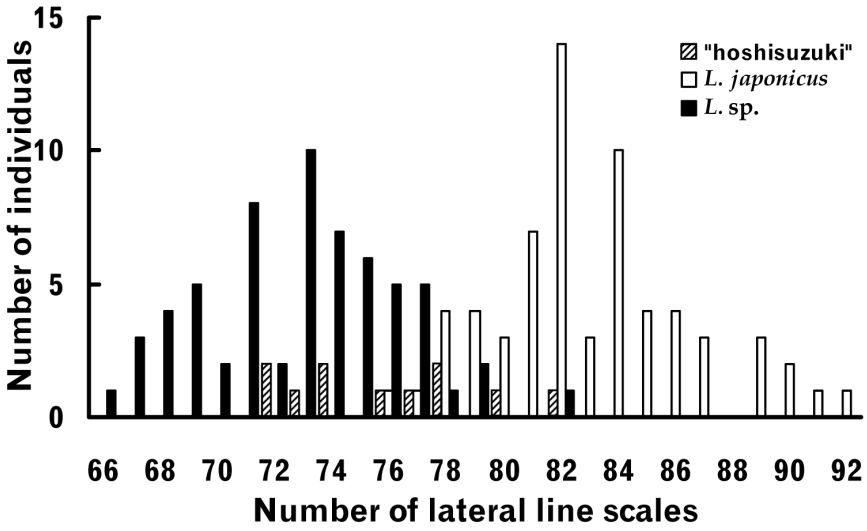

Fig. 15. Frequency distributions of lateral line scales (LLS) in the "hoshisuzuki", ***Lateolabrax japonicus*** and ***L.*** sp.

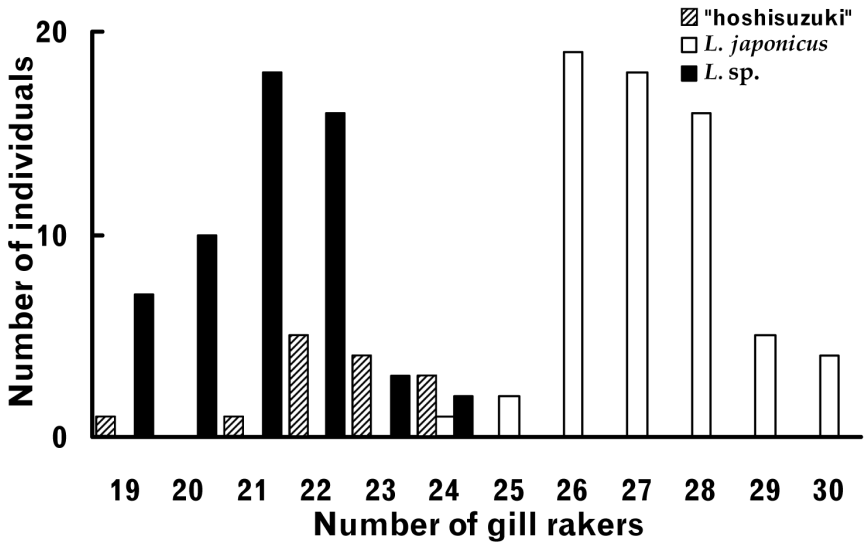

Fig. 16. Frequency distribution of total gill rakers (GR) in the "hoshisuzuki", ***Lateolabrax japonicus*** and ***L***. sp.

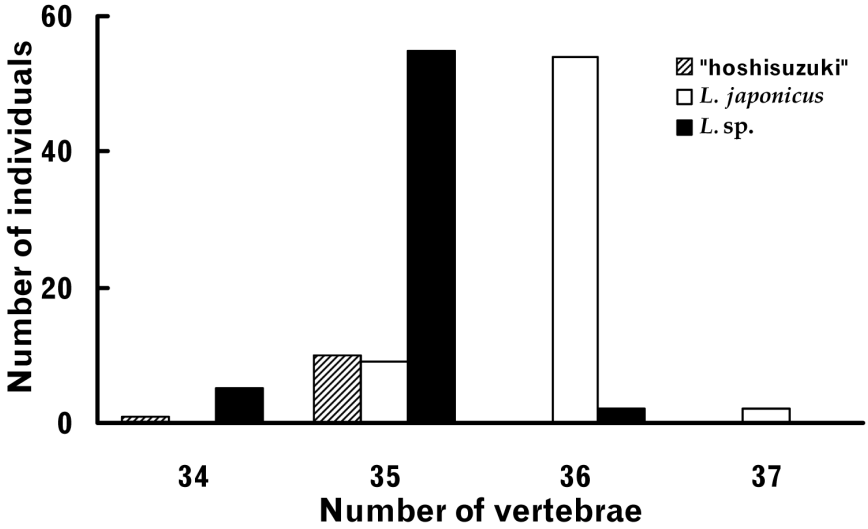

Fig. 17. Frequency distribution of vertebrae (VT) in the "hoshisuzuki", ***Lateolabrax japonicus*** and ***L***. sp.

of LLS and GR in "hoshisuzuki" were almost within the range of ***L***. sp., although they inclined somewhat towards the right from the modes of normal distributions of ***L***. sp. (Figs. 15, 16).

The modes of vertebral (VT) frequencies in ***L***. ***japonicus*** and ***L***. sp. were explicitly separated, that is, 36 and 35, respectively (Fig. 9). In the 10 individuals of "hoshisuzuki" for which vertebrae could be counted, 9 individuals had 35 and 1 individual had 34, being within the range of ***L***. sp. (Fig. 17).

Further, the squamation on the dorsal surface of the head (Fig. 11) was examined. An individual whose number is T-1 corresponded with the feature of ***L***. sp. (Fig. 11 B), while the other individuals corresponded with the feature of ***L***. ***japonicus*** in which the scale rows extended anteriorly beyond the nostrils (Fig. 11 A).

Table 8. Genotypes of "hoshisuzuki" and non-spotted sea bass (genus ***Lateolabrax***)

Individual Nc	T-1	F-97	U-2	U-3	U-4	U-5	U-6	U-7	U-8	U-10	U-11	U-12	U-13	O-1	F-98	F-99	U-1	U-9
Type[1]	H	H	H	H	H	H	H	H	H	H	H	H	H	H	N	N	N	N
AAT-1*	AA	AA	AC	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AB	AB	AA	AB	AA
AAT-2*	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
ADH*	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AB	AA	BB	AA
CK*	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
G3PDH-1*	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
G3PDH-2*	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
GPI-1*	BB	BB	BB	BC	BB	BB	BC	BB	BB	BC	BB	BB	AB	BB	AA	AA	AB	AA
GPI-2*	AA	AB	AC	AA	AA	AA	AA	AA	AA	AA	AB	AA	AA	AA	AA	AA	AB	AA
IDDH*	AA	AA	AA	AA	AC	AA	AB	AA	AA	AA	AC	AA	AA	AA	AA	AC	AA	AA
IDHP-1*	AB	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
LDH-2*	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	AA	AA	AA	AA
MDH-1*	AA	AA	AB	AA	AA	AA	AB	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
MDH-2*	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
MEP*	CC	AC	AA	AA	AC	AA	AA	AA	AA	AA	AA	AA	BB	AA	AA	AA	AA	AA
MPI-1*		AB	AB	AB	AB	AB	AA	AB	BB	AB	AA	AA	AA	AB	AA	AA	AB	AB
MPI-2*		AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
PGDH*	AA	AA	AA	AB	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
PGM*	AA	AB	AA	BB	BB	BB	AB	AB	AC	BB	AB	BC	AA	AB	AB	AA	AA	AB
PROT-1*	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	AA	AA	AA	AA
SOD-1*	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA

[1] H: "Hoshisuzuki"; N: Non-spotted sea bass

Alleles are symbolized as capital letters, the most dominant allele (*100 or *-100) in ***L .. japonicus*** being A. B and C indicate alleles of lessening frequenc

Results of genetic characters

Twenty isozymic loci were examined, as in section 3, all genotypes at each locus of the individuals examined being shown in Table 8. The loci in which significant differences in allelic composition between ***L. japonicus*** and ***L.*** sp. were observed (Fig. 12) are described as follows.

The ***ADH**** locus of "hoshisuzuki" was monomorphic, being occupied by the ****-100*** (A) allele, the same as that of ***L.*** sp., while a few individuals of the non-spotted type were polymorphic (Table 8). The features of "hoshisuzuki" and the non-spotted type corresponded well with the relationship between the two species (Table 5).

The ***GPI-1**** locus of "hoshisuzuki" was comprised mainly of the ****110*** (B) allele, like that in ***L.*** sp., whereas that of the non-spotted type was comprised mainly of the ****100*** (A) allele, like that in ***L. japonicus*** (Table 8).

At the ***LDH-2**** locus of "hoshisuzuki", only the ****100*** (B) allele which characterizes ***L.*** sp. appeared, whereas at that of the non-spotted type, only the ****-100*** (A) allele which characterizes ***L. japonicus*** appeared (Table 8).

At the ***PROT-1**** locus of "hoshisuzuki", only the ****170*** (B) allele which characterizes ***L.*** sp. appeared, whereas at that of the non-spotted type, only the ****100*** (A) allele which characterizes ***L. japonicus*** appeared (Table 8). The features of "hoshisuzuki" and the non-spotted type at this locus corresponded completely with the relationship between the former two species (Table 5).

Allelic frequencies were calculated for all of the individuals of "hoshisuzuki" and the non-spotted type, respectively. For a comparison, the data of ***L. japonicus*** and ***L.*** sp. (Table 5) were added and genetic distances (D values) after Nei (1972) were calculated from the allelic frequencies of the four groups. Unbiased values for each D value were calculated after Nei (1978) and standard errors according to

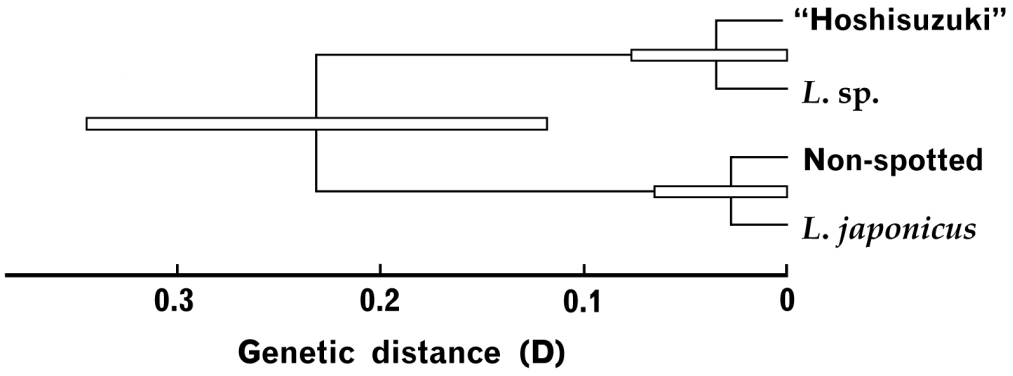

Fig. 18. Dendrogram based on unbiased genetic distances among "hoshisuzuki", ***Lateolabrax*** sp., non-spotted sea bass and ***L. japonicus***. Open bars indicate standard errors.

each unbiased D value were calculated. Subsequently, a dendrogram based on the unbiased genetic distances with the standard errors was made by the UPGMA method (Fig. 18). The "hoshisuzuki" and ***L***. sp., and the non-spotted type and ***L***. ***japonicus*** formed a single cluster in a close distance, respectively, the distance between the two clusters having quite a similar value to that between ***L***. ***japonicus*** and ***L***. sp., reported in section 3.

Discussion

Although "hoshisuzuki" corresponded well with the features of ***L***. sp. in many morphological characters, some characters showed no correspondence. The PSAL values of "hoshisuzuki" were similar to those of ***L***. ***japonicus***, showing that it has a larger scaly area than that of ***L***. sp. (Table 7). However, PSAL examined in section 3 was for juveniles, of which the total length was around 100mm (Table 2). Because most of the "hoshisuzuki" examined in this section were much larger than those, it might be reasonable to consider that PSAL increases relatively with growth.

In the meristic characters, the tendency for the counts of LLS and GR to shift towards the right from the modes of normal distributions of ***L***. sp. was recognized (Figs. 15, 16). For this reason, it is probable that the counts of LLS and GR increase with growth. The meristic characters are constant and generally do not change after the juvenile stage. However in the case of the sea bass, it is occasionally observed that some fine scales exist on an extension of the lateral line, and some traces of gill rakers exist at the joint parts between the upper and lower limbs of gill arches and the body. The probability that such traces which are not included in the counts in the larval and juvenile stages will be tangible can be considered, therefore the fact that the counts of LLS and GR shift towards the right from the modes of ***L***. sp. may be caused by these factors. However, even considering these matters, the LLS and GR counts are available to identify ***L***. ***japonicus*** and ***L***. sp. because the values in those characters of "hoshisuzuki" are within the ranges of ***L***. sp.

The squamation on the dorsal surface of the head corresponded with the feature of ***L***. ***japonicus*** except for one individual (T-1). Because T-1 is a small individual whose total length is less than 200mm (Table 7), it could correspond well with the features of the ***L***. sp. fingerlings. While the other individuals are larger than that, it may be significant to consider that the squamation changed with growth, and that extended scale rows were newly formed before the anterior nostrils. This is common to PSAL as stated earlier, and it was considered that some characters of ***L***. sp. including some meristic characters previously referred to change with growth. For this matter, further detailed study is needed.

Although "hoshisuzuki" showed differences from ***L***. sp. in some of the morphological characters, those can be regarded as differences in the growth stages. In the genetic characters, they corresponded well with the features of ***L***. sp. (Table 8), and they were genetically regarded as the same as ***L***. sp. Those factors suggest that the so-called "hoshisuzuki" are adult individuals of the Chinese sea bass ***Lateolabrax*** sp., which were imported as aquacultural seeds and escaped from their cages.

The probability of the effects of ***L***. sp. escaping into Japanese waters is of considerable concern; in particular, hybridization with the native ***L***. ***japonicus*** is worrying. The spawning season of ***L***. sp. in the Bohai Sea of China is reported to be from August to November (Liu and Qin, 1987; Deng et al., 1988; Jiang et al., 1988; Wan and Chen, 1988), being different from the general spawning season of ***L***. ***japonicus*** in Japan, which is from October to March (Ochiai and Tanaka, 1986); however, a spawning ecology in a different environment cannot be expected at all.

In the Harima Sea of Kagawa Prefecture, ***L***. ***japonicus,*** which migrate to spawn, are abundantly caught by trawling and so on from December to January of every year. The author sometimes observed some large individuals which are obviously presumed to be ***L***. sp. in appearance among the spawning schools at fish markets. Although this fact directly suggests that hybridization does occur, the probability is likely enough. For the problem of hybridization between the two species, monitoring should be done hereafter.

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5 Morphological and genetic characteristics of *Lateolabrax japonicus* from the Ariake Sea, Japan

Based on morphological and genetic differences observed in Japanese and Chinese sea bass, it was concluded that the Chinese sea bass was specifically distinct from ***Lateolabrax japonicus***, the former being referred to as ***Lateolabrax*** sp. Subsequently, Yokogawa and Tajima (1996) proposed the common English name "spotted sea bass" for ***L***. sp. Further, Nakayama et al. (1996) recognized significant morphological differences in developing larvae of the two species.

L. sp. is characterized by many clear black dots on the lateral body region. Sea bass from the Ariake Sea, Japan are also known to have similar external features (Katayama, 1960a, b, 1965). Therefore at this section, morphological and genetic characteristics of sea bass from the Ariake Sea were examined.

Materials and methods

Sea bass from the Ariake Sea (hereafter called Ariake form) used at this section were obtained offshore from Shimabara City, Nagasaki Prefecture, on 18th－26th, May, 1993. Forty specimens (345－535mm TL, average 420.6mm TL) caught by hook and line were used for the morphological and genetic analyses. The specimens were morphologically and genetically examined with the common methods noted at section 2. As the genetic markers, 20 isozymic loci were used as same as at sections 3 and 4.

The specimens examined at this section have been deposited in the Kanagawa Prefectural Museum of Natural History (KPM-NI0009696－0009714), Faculty of Science, Kochi University (BSKU 57885－57905) and Tokushima Prefectural Museum (TKPM-P 6096－6099).

Results of morphological characters

Average values and ranges of the proportions of length-measured characters of the Ariake form, together with the average values of Japanese and Chinese species examined previously (hereafter called ***Lateolabrax japonicus*** and ***L***. sp., respective-

Table 9. Proportions of body characters in the Ariake form, ***Lateolabrax japonicus*** and ***L*** . sp.

	Ariake		***L*** . ***japonicus*** [1]	***L*** . sp.[1]
	Average	Range	Average	Average
Total length[2]	119.16	115.13 — 122.60	121.69	119.17
Fork length[2]	114.00	111.28 — 117.18	115.11	114.00
Pre-anus length[2]	65.41	62.18 — 73.00	66.04	66.76
Body depth[3]	23.09	21.01 — 27.58	24.24	26.28
Body width[2]	13.32	11.68 — 16.25	13.38	13.46
Caudal peduncle depth[2]	9.15	8.22 — 10.73	9.28	10.35
Caudal peduncle length[2]	22.36	20.49 — 24.36	21.61	22.09
Pre-dorsal length[2]	34.52	32.67 — 36.79	35.24	35.00
First dorsal fin length[2]	14.30	12.31 — 15.55	14.10	12.62
Second dorsal fin length[2]	10.69	8.07 — 13.50	11.82	12.57
Anal fin length[2]	11.69	9.61 — 13.72	12.49	14.33
Pectoral fin length[2]	16.10	14.66 — 19.47	17.05	16.08
Pelvic fin length[2]	16.84	15.00 — 19.01	17.57	18.33
Head length[2]	30.60	28.96 — 32.69	31.98	32.56
Snout length[3]	26.59	24.14 — 28.57	26.25	25.10
Orbital diameter[3]	16.59	14.49 — 19.01	17.70	24.83
Interorbital width[3]	22.30	20.33 — 23.87	21.20	21.64
Suborbital width[3]	10.39	8.55 — 12.17	11.26	10.72
Upper jaw length[3]	43.05	40.00 — 45.98	42.36	44.21
Lower jaw length[3]	46.99	43.10 — 49.95	46.43	46.82
Pectoral scaly area length[4]	29.00	19.96 — 41.26	26.73	19.43

[1] Data from Table 3
[2] Percentage of standard length
[3] Percentage of head length
[4] Percentage of pectoral fin length

Table 10. Meristic counts in the Ariake form, ***Lateolabrax japonicus*** and ***L*** . sp.

	Ariake		***L*** . ***japonicus*** [1]	***L*** . sp.[1]
	Average	Range	Average	Average
Dorsal fin spines	13.00	12 — 14	12.85	12.95
Dorsal fin soft rays	12.48	11 — 13	12.78	13.07
Anal fin spines	3.00	3 — 3	3.00	2.98
Anal fin soft rays	7.23	6 — 8	7.73	7.53
Pectoral fin soft rays	16.90	16 — 18	16.85	16.31
Pelvic fin spines	1.00	1 — 1	1.00	1.00
Pelvic fin soft rays	5.00	5 — 5	5.00	5.00
Pored scales on lateral line	80.75	75 — 88	83.08	72.86
Scales above lateral line	16.00	14 — 18	14.85	15.82
Scales below lateral line	19.40	16 — 22	19.37	19.18
Gill rakers (upper limb)	8.43	6 — 11	9.66	6.38
Gill rakers (lower limb)	17.80	15 — 21	17.52	14.70
Gill rakers (total)	26.23	23 — 30	27.19	21.07
Vertebrae	35.58	33 — 37	35.89	34.95

[1] Data from Table 4

ly) are shown in Table 9. Average values of the Ariake form were close to either ***L. japonicus*** or ***L***. sp. in some characters, but distinctly different to both in others.

Thus, no general tendency in these characters for the Ariake form was apparent. The average value of the pectoral scaly area length (PSAL) in the Ariake form was greater than in ***L. japonicus*** and ***L.*** sp.

Average values and ranges of meristic counts in the Ariake form, together with the average values for ***L. japonicus*** and ***L.*** sp. are shown in Table 10. Although the average values for the former tended to be close to those for ***L. japonicus***, the range of some character counts was intermediate between ***L. japonicus*** and ***L.*** sp.

Figures 19−21 show frequency distributions of the number of pored lateral line scales (LLS), number of total gill rakers (GR) and number of vertebrae (VT), respectively, in the Ariake form, together with data for ***L. japonicus*** and ***L.*** sp.

The histogram of LLS in the Ariake form indicated a bimodal pattern, the concavity between the peaks corresponding closely to the border between ***L. japonicus***

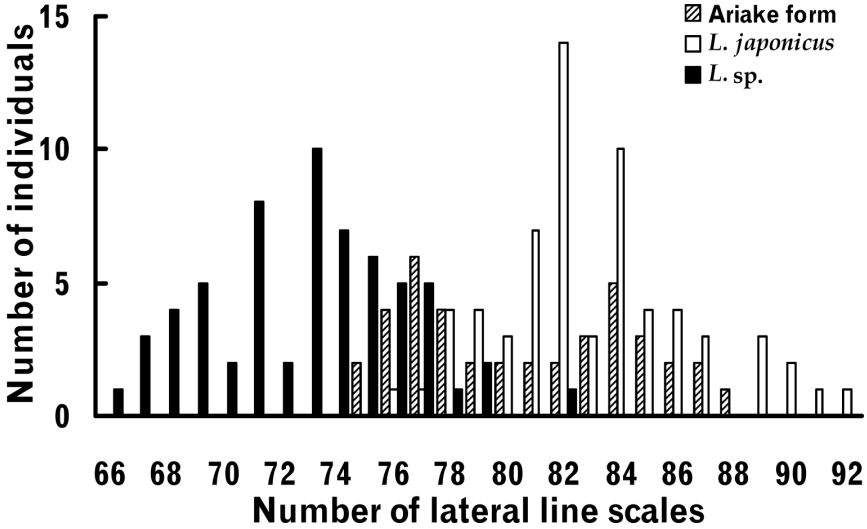


Fig. 19. Frequency distributions of lateral line scales (LLS) in the Ariake form, ***Lateolabrax japonicus*** and ***L.*** sp.

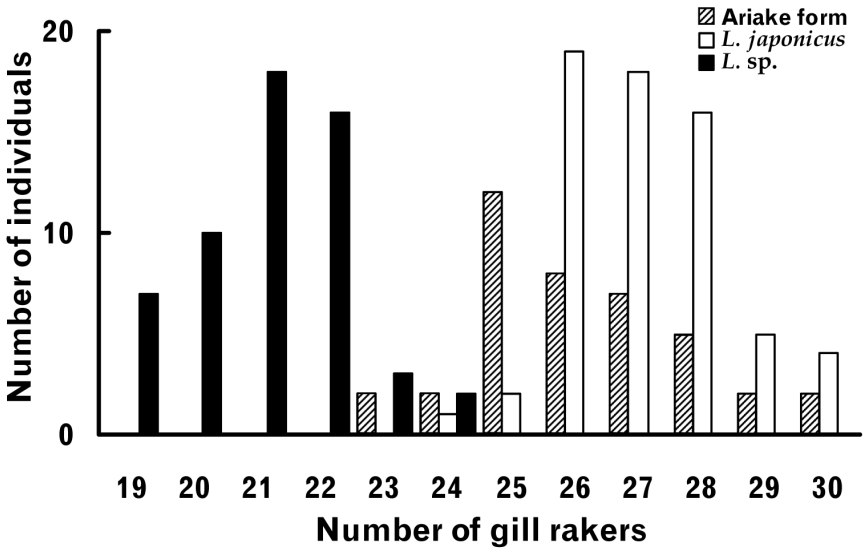


Fig. 20. Frequency distributions of total gill rakers (GR) in the Ariake form, ***Lateolabrax japonicus*** and ***L.*** sp.

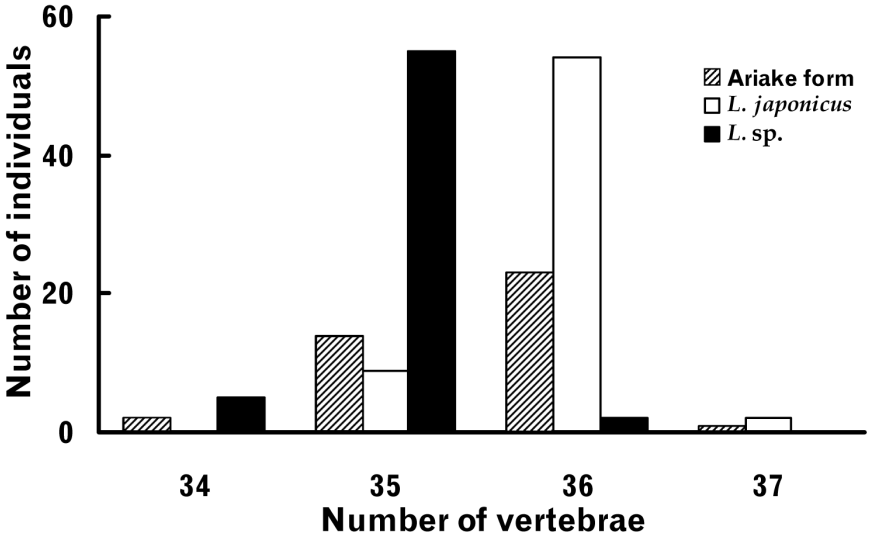


Fig. 21. Frequency distributions of vertebrae (VT) in the Ariake form, ***Lateolabrax japonicus*** and ***L***. sp.

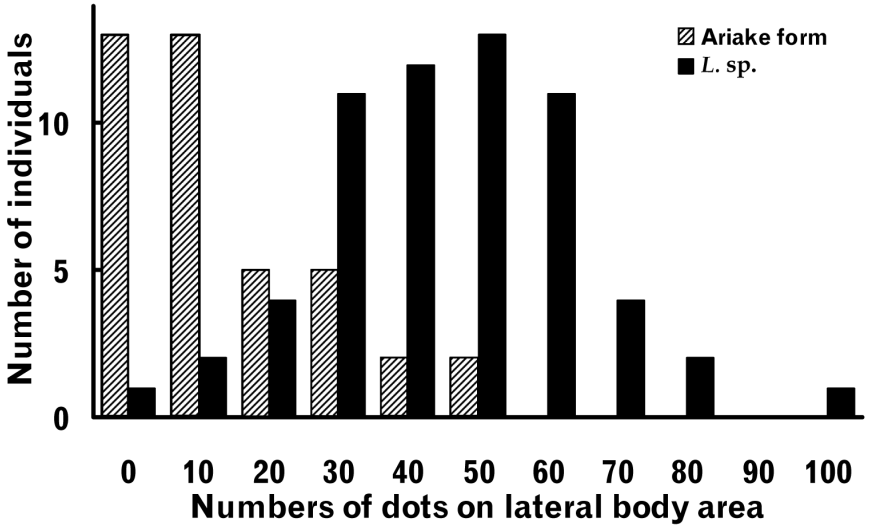


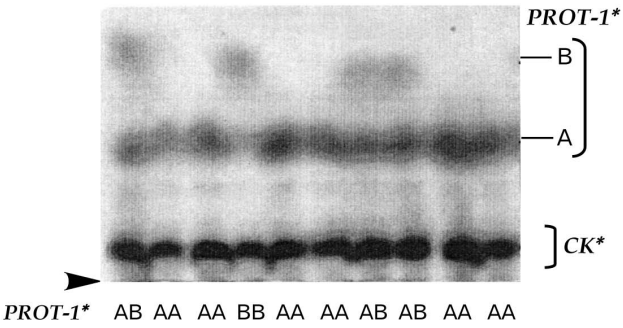
Fig. 22. Frequency distributions of number of dots on the lateral body area in the Ariake form and ***Lateolabrax*** sp.

and ***L***. sp. The range of LLS was 75—88, intermediate between the two species (Fig. 19), although inclined somewhat towards ***L***. ***japonicus***.

The histogram of GR in the Ariake form (modal number 25) corresponded closely to that of L. japonicus (modal number 26) (Fig. 20). GR range in the Ariake form was 23—30, slightly overlapping that of ***L***. sp.

The modes of vertebral (VT) frequencies in ***L***. ***japonicus*** and ***L***. sp. were explicitly separated, that is, 36 and 35, respectively. The histogram of the Ariake form indicated a mode of 36, which, however, was not as dominant as those for ***L***. ***japonicus*** or ***L***. sp., (40.0% of the total had fewer than 36 vertebrae, a characteristic of ***L***. sp.). The range of VT in the Ariake form, 34-37, wholly included those of the two species (Fig. 21).

The lateral body dot numbers in the Ariake form, together with data for ***L***. sp.,



(Upper case letters represent genotypes;
in ***PROT-1**** locus; A: ***100*** allele, B: ***170*** allele)

Fig. 23. Electrophoretogram of general protein in the Ariake form. Upper case letters represent genotypes, at ***PROT-1**** locus; A: ****100*** allele, B: ****70*** allele.

are shown in Fig. 22. In the Ariake form, 67.5% of the total possessed dots, the most dominant dot numbers being 10—20 (ranging 0 to about 60). Although the presence of dots on the lateral body surface is a typical character of the Ariake form, the shape of the histogram differed somewhat from that for ***L***. sp., which is also characterized by dots (Fig. 22).

Dorsal squamation on the head was examined, all of the Ariake specimens having well-developed scale rows from the interorbit to the internasal area. The rows subsequently extended anteriorly over the nostrils as in ***L. japonicus***.

Results of genetic characters

At the ***PROT-1**** locus, at which alleles are totally replaced between ***L. japonicus*** and ***L***. sp. (Table 5), monomeric heterozygotes appeared frequently (Fig. 23). The fitness of the allelic frequencies at polymorphic loci, including ***PROT-1****, according to Hardy-Weinberg equilibria was examined by chi-square tests (Table 11). Because no χ^2 values were significant at the 5% level, the Ariake form was regarded as having originated from a simple Mendelian population.

Table 11. Fitness for Hardy-Weinberg equilibrium by chi-square tests of polymorphic loci in the Ariake form

Locus	Alleles	d.f. [1]	chi-square	P [2]
AAT-1*	3	3	0.686	0.877
ADH*	2	1	1.246	0.264
G3PDH-1*	2	1	0.006	0.938
G3PDH-2*	2	1	0.009	0.924
GPI-1*	2	1	3.358	0.067
GPI-2*	2	1	0.062	0.803
IDDH*	3	3	0.681	0.878
IDHP-1*	2	1	0.027	0.870
LDH-2*	2	1	0.001	0.975
MDH-1*	2	1	0.121	0.728
MEP*	3	3	0.111	0.991
MPI-1*	2	1	1.183	0.277
PGDH*	3	3	0.026	0.999
PGM*	3	3	6.060	0.109
PROT-1*	2	1	0.580	0.446
SOD-1*	2	1	0.006	0.938

[1] Degrees of freedom

[2] Risk percentage of chi-square value

Average allele numbers per locus, rate of polymorphic loci and average heterozygosity (average rate of heterozygous loci per individual) of the Ariake form, together with values for ***L. japonicus*** and ***L***. sp. are shown in Table 12. Compared with ***L. japonicus*** and ***L***. sp., the values of these genetic features in the Ariake form were somewhat higher. In addition, the Ho/He ratio in the Ariake form was greater than 1, indicating an ex-

Table 12. Genetic features of the Ariake form, ***Lateolabrax japonicus*** and ***L*** . sp.

		Ariake	***L*** . ***japonicus*** [1]	***L*** . sp.[1]
Alleles/Locus		2.100	1.826	1.850
P*		0.500	0.478	0.350
P		0.300	0.130	0.250
P+P*		0.800	0.609	0.600
Average	Ho	0.170	0.113	0.105
Heterozygosity	He	0.158	0.136	0.125
	Ho/He	1.074	0.829	0.840

[1] Data based on the secton 3

P*: Polymorphism less than 0.95%, P: Polymorphism over 0.95%

Ho: Observed heterozygosity, He: Expected heterozygosity

cess of heterozygotes, unlike the situation in ***L***. ***japonicus*** or ***L***. sp. in which excesses of homozygotes were found (Table 12). This showed the Ariake form as having high genetic variation.

The frequency distribution of heterozygosity (rate of heterozygous loci in each individual) in the Ariake form is shown in Fig. 24, being a plain binomial distribution. If F1 hybrids existed in the population, a typical convexity should be recognizable in the high heterozygosity zone (Taniguchi et al., 1985; Macaranas et al., 1986; Yokogawa, 1996). In the present population, however, no evidence of this was apparent.

Allelic frequencies of dotted and non-dotted individuals from the Ariake Sea are shown in Table 13. Although differences in the allelic frequencies between them, following chi-square tests, were not significant because of the low sample numbers, differences were recognized at the ***GPI-1****, ***LDH-2****, and ***PROT-1**** loci, at which great differentiation has been detected between ***L***. ***japonicus*** and ***L***. sp. (Table 5). The allelic compositions of these loci in the dotted type had shifted towards those in ***L***. sp.

Regarding the pooled allelic frequencies of the Ariake form, at the ***PROT-1**** locus the frequency of the ****170*** allele, which occupies the locus in ***L***. sp., was 0.213. Although the frequency was therefore closer to that of ***L***. ***japonicus***, the value nev-

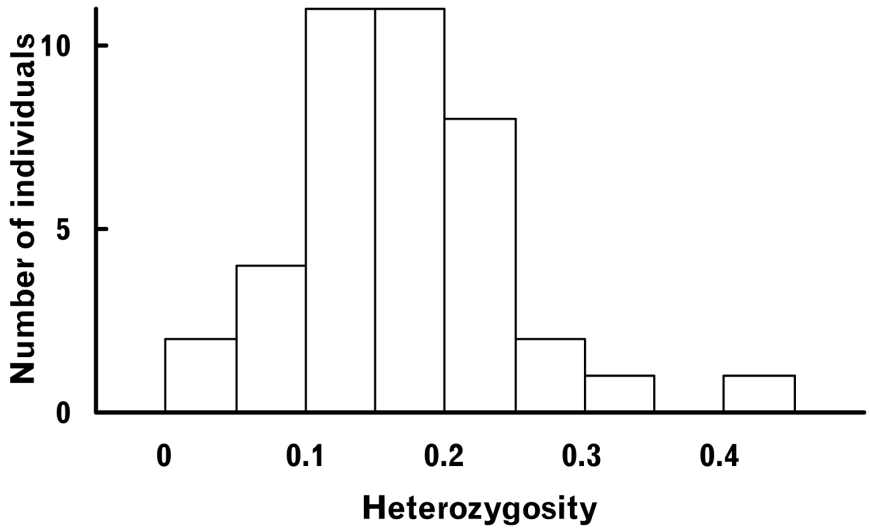


Fig. 24. Frequency distribution of heterozygosity (rate of heterozygous loci in each individual) in the Ariake form.

Table 13. Allelic frequencies of the Ariake form (dotted and non-dotted types), ***Lateolabrax japonicus*** and ***L*** . sp.

Locus	Allele	Ariake			*L . japonicus*	*L* . sp.[1]
		Dotted	Non-dotted	Pooled		
*AAT-1**	***100**	0.865	0.917	0.882	0.792	0.981
	***85**	0.135	0.042	0.105	0.208	0.019
	***65**	0.000	0.042	0.013	0.000	0.000
*AAT-2**	***-100**	1.000	1.000	1.000	1.000	1.000
*ADH**	***-50**	0.000	0.000	0.000	0.011	0.000
	***-100**	0.833	0.885	0.850	0.659	1.000
	***-150**	0.167	0.115	0.150	0.330	0.000
*CK**	***100**	1.000	1.000	1.000	1.000	1.000
*G3PDH-1**	***100**	0.981	1.000	0.988	1.000	1.000
	***85**	0.019	0.000	0.013	0.000	0.000
*G3PDH-2**	***100**	0.972	1.000	0.982	0.986	1.000
	***-200**	0.028	0.000	0.018	0.014	0.000
*GPI-1**	***130**	0.000	0.000	0.000	0.000	0.060
	***110**	0.278	0.231	0.263	0.100	0.922
	***100**	0.722	0.769	0.737	0.900	0.018
*GPI-2**	***-100**	0.942	1.000	0.962	0.937	0.940
	***-250**	0.058	0.000	0.038	0.063	0.060
*IDDH**	***165**	0.096	0.154	0.115	0.037	0.200
	***100**	0.885	0.846	0.872	0.963	0.800
	***-50**	0.019	0.000	0.013	0.000	0.000
*IDHP-1**	***120**	0.019	0.038	0.026	0.000	0.000
	***100**	0.981	0.962	0.974	0.912	0.588
	***70**	0.000	0.000	0.000	0.088	0.412
*LDH-2**	***100**	0.259	0.154	0.225	0.097	0.966
	***-100**	0.741	0.846	0.775	0.903	0.034
*MDH-1**	***100**	0.960	0.917	0.946	0.986	1.000
	***70**	0.040	0.083	0.054	0.014	0.000
*MDH-2**	***-100**	1.000	1.000	1.000	1.000	1.000
*MEP**	***150**	0.037	0.038	0.038	0.079	0.050
	***100**	0.944	0.962	0.949	0.886	0.900
	***50**	0.019	0.000	0.013	0.035	0.050
*MPI-1**	***125**	0.357	0.444	0.383	0.306	0.363
	***100**	0.643	0.556	0.617	0.694	0.638
*MPI-2**	***100**	1.000	1.000	1.000	1.000	0.987
	***75**	0.000	0.000	0.000	0.000	0.013
*PGDH**	***120**	0.000	0.038	0.013	0.000	0.011
	***100**	0.981	0.962	0.974	1.000	0.989
	***55**	0.019	0.000	0.013	0.000	0.000
*PGM**	***115**	0.000	0.000	0.000	0.021	0.060
	***100**	0.370	0.346	0.363	0.465	0.500
	***75**	0.611	0.654	0.624	0.507	0.431
	***55**	0.019	0.000	0.013	0.007	0.009
*PROT-1**	***170**	0.259	0.115	0.213	0.000	1.000
	***100**	0.741	0.885	0.787	1.000	0.000
*SOD-1**	***145**	0.000	0.038	0.013	0.000	0.000
	***100**	1.000	0.962	0.987	1.000	0.964
	***20**	0.000	0.000	0.000	0.000	0.036

[1] Data from Table 5

ertheless fell between those for ***L. japonicus*** and ***L.*** sp. Similarly, the pooled allelic frequencies at the ***AAT-1****, ***ADH****, ***GPI-1****, ***IDDH**** and ***LDH-2**** loci of the Ariake form fell between those of the two species.

Discussion

Regarding morphological characters, although the Ariake form showed a range between ***Lateolabrax japonicus*** and ***L.*** sp. in some of the meristic counts, no general tendency in body proportions was apparent. Because some sea bass proportions are known to change with growth (for example, orbital diameter decreases with growth [Fig. 3]), exact comparisons of proportions should be made on similarly-sized specimens. At this stage, because morphological characters, including PSAL and head scale development, may also change with growth, further comments on morphological characters cannot be made.

On the other hand, the meristic counts are considered to be generally stable with growth. Therefore, the LLS and GR counts in the Ariake form, which showed intermediate ranges between ***L. japonicus*** and ***L.*** sp., can be considered to reflect the former's genetic properties.

As for the genetic characters, pie graphs of allelic frequencies reported in some sea bass populations, including ***L.*** sp., are shown in Fig. 25. Loci included are ***ADH****, ***GPI-1****, ***LDH-2**** and ***PROT-1****, in which considerable differences have been recognized between ***L. japonicus*** and ***L.*** sp. Although the allelic compositions tended to vary by population in ***L. japonicus***, it was clear that the allelic frequen-

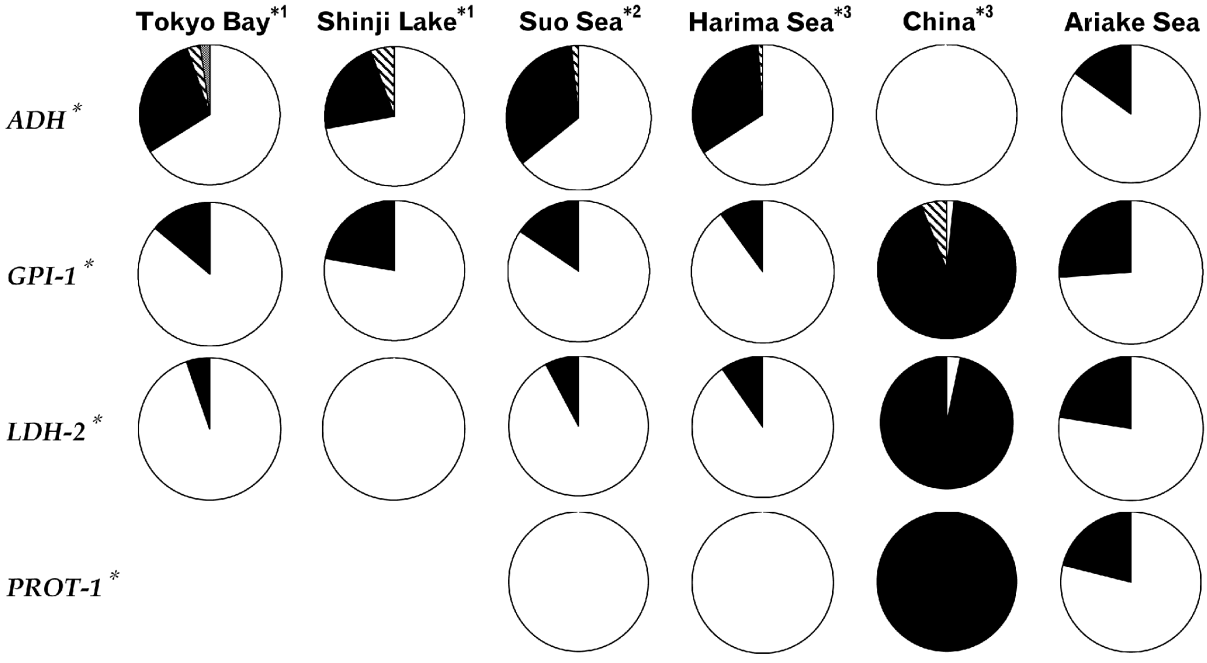


Fig. 25. Pie graph comparisons of allelic frequencies at significant loci in selected sea bass populations. Allelic frequencies given in Table 4. From *1 Tsuda (1989); *2 Table 21; *3 Table 5.

cies of the Ariake form differed from the other ***L. japonicus*** populations.

Although the allelic composition of the Ariake form was generally closer to ***L. japonicus*** rather than to ***L.*** sp., it shifted towards that of ***L.*** sp. at the loci noted above (Fig. 25), suggesting that the Ariake form has been genetically influenced by ***L.*** sp. However, such has not occurred recently, since there was no evidence of F1 hybrids (Fig. 24).

L. sp. influence was also apparent in the LLS and GR characters. The occurrence of dots, which is a specific character of ***L.*** sp., can also be genetically affected, apparently by the same mechanism as operating in ***L. japonicus*** populations (see section 6).

Figure 26 shows a dendrogram of genetic distances (D values), after Nei (1972), made by the UPGMA method utilizing the sea bass populations shown in Fig. 25. Although a criticism, that the numbers of loci based on the D values between the populations were not uniform, might be valid, the dendrogram shape could not have changed very much, even if unexamined allelic frequency data were added, because the allelic compositions of ***L. japonicus*** populations examined by Tsuda (1989) were similar to those found at section 3. According to the figure, ***L. japonicus*** populations, except the Ariake form, formed close clusters, the Ariake form being separated from the former by a genetic distance of nearly 0.01 (subspecific level according to Menezes et al. [1990]).

Kinoshita et al. (1995) examined the morphometrics of larval ***L. japonicus*** from various localities around the Japanese archipelago, finding that the larval Ariake form was significantly different from other ***L. japonicus*** populations. They considered that the Ariake form was a relict population from the Asian continent, such an opinion being given some support by the present genetic evidence.

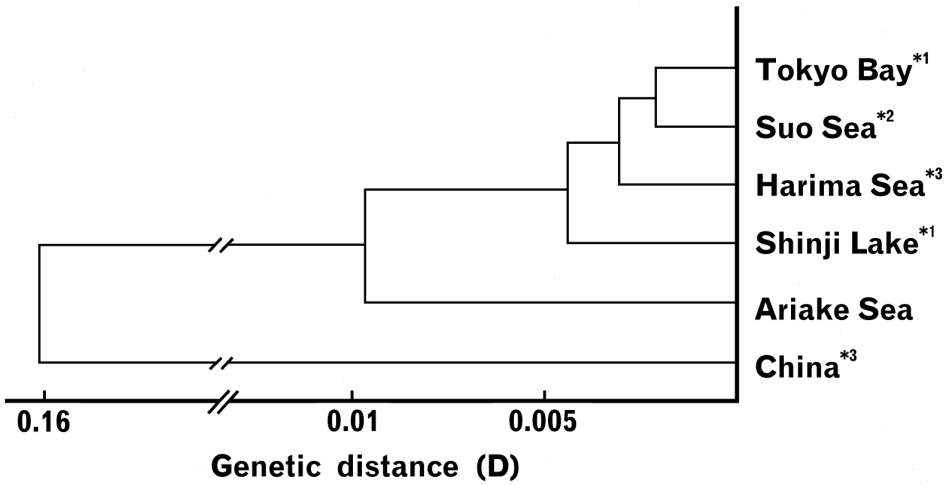


Fig. 26. Genetic distance dendrogram for selected sea bass populations. From *1 Tsuda (1989); *2 Table 21; *3 Table 5. Allelic frequencies at 20 loci, examined in section 3, and 13 loci, not including ***PROT-1*** examined by Tsuda (1989) were used for the calculation of D values.

In any case, it can be concluded that the Ariake form is independent of other ***L. japonicus*** populations. Several hypotheses regarding its origin are considered as follows:

Hypothesis 1: ***L***. sp. has been introduced into the Ariake Sea artificially, resulting in introgressive hybridization.

Hypothesis 2: During the divergence of ***L. japonicus*** and ***L***. sp. from a common ancestor, an intermediate form has been isolated in the Ariake Sea.

Hypothesis 3: Following the divergence of ***L. japonicus*** and ***L***. sp., the latter secondarily entered the Ariake Sea on single or some occasions, resulting in introgressive hybridization.

Hypothesis 4: After the specific divergence of ***L. japonicus*** and ***L***. sp., population mixing occurred by diastrophism, resulting in introgressive hybridization over a wide area. Thereafter, a hybridized population has been isolated in the Ariake Sea.

Although the relative merits of the hypotheses are difficult to determine, hypotheses 3 and 4 may be the most reasonable.

The Ariake Sea is considered to be a specialized region, including some peculiar animal species and an overall faunal affinity with the Asian continent (Sugano, 1981; Washio et al., 1996). Menezes et al. (1990) examined genetic divergence in some sciaenid fishes, including specimens from the Ariake Sea, finding that the Ariake Sea population of ***Nibea albiflora*** was distinct from the other populations of that species. The genetic divergence of the Ariake Sea population of ***N. albiflora*** was supported by significant differences in morphological characters (Takita, 1974), not unlike the situation for the Ariake form of ***L. japonicus*** found in this study.

Furthermore, it is known that the populations of bluespotted mud hopper, ***Boleophthalmus pectinirostris***, and constricted tagelus, ***Sinonovacula constricta***, which are typical faunal components of the Ariake Sea, are genetically divergent from populations on the continent (Furukawa et al., 1996; Y. Natsukari, pers. comm.). Such genetic divergences are also supported by significant differences in morphological characters (Koga, 1993; Yoshimoto, 1994).

Such evidence suggests that the faunal population in the Ariake Sea has been isolated for a considerable time; that is, that the Ariake Sea itself has been isolated from other water bodies.

In the case of the sea bass, it appears reasonable to propose that ***L. japonicus*** and ***L***. sp. populations became mixed at some time, the resulting introgressively-hybridized population being subsequently isolated in the Ariake Sea. This possibility is supported by the considerable genetic variation in the Ariake form (Table

12).

It may be significant that theoretical hybridization of ***L***. sp. with ***L***. japonicus with a rate of 0.213 (the ****170*** allele frequency at the ***PROT-1**** locus in the Ariake form [Table 13]), results in expected allelic frequencies at loci for the markers shown in Figure 25, being fairly close to those found in the Ariake form.

However, the theoretical allelic frequencies at some other loci, such as ***IDHP-1**** and ***PGM****, do not correspond with those of the Ariake form. To explain this conflict, some possibilities were considered. It is unknown at this time when introgressive hybridization occurred. Should the allelic frequencies at some loci of either or both species at that time have differed from those of the modern forms, the hybridized allelic frequencies would also have differed from the theoretical values given here.

On the other hand, some genetically-independent populations of ***L***. sp. may also exist, with a population distinct from the specimens examined at section 3 hybridizing with ***L***. ***japonicus***. In fact, Yokogawa and Tajima (1996) reported that the Taiwanese form of ***L***. sp. was somewhat divergent both morphologically and genetically from the Chinese form, supporting the above suggestion. However, more information, not only supporting hybridization but also clarifying its process exactly is necessary.

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Dr. Hiroshi Senou, Kanagawa Prefectural Museum of Natural History, Dr. Kunio Sasaki, Faculty of Science, Kochi University, and Dr. Yoichi Sato, Tokushima Prefectural Museum, accepted the specimens used at this section as official specimens. Finally, I wish to thank Mr. Roger Ahlberg, Japan English Service, Inc., Chiba, for checking the manuscript.

6 Morphological and genetic features of *Lateolabrax japonicus* with black dots on lateral body region, including such individuals in the Ariake population

In the last section, the morphological and genetic characteristics of sea bass from the Ariake Sea, which are similar to ***Lateolabrax*** sp. in appearance, were examined. And it was suggested that the Ariake form is a specialized population originating from the hybridization of ***L. japonicus*** and ***L.*** sp, while it is also well known that young individuals of ***L. japonicus*** have a few small black dots on the lateral body region like the sea bass from the Ariake Sea (Fig. 27) (Katayama, 1965a; Miyadi et al., 1976; Kawamura, 1989).

Therefore, in this section, the morphological and genetic characteristics of such dotted types of Japanese sea bass were compared with those of ***L. japonicus*** and ***L.*** sp. Also, in regard to the Ariake population examined earlier, they were separated into dotted and non-dotted types and the same comparison was carried out.

Materials and methods

Data from specimens used for this section are shown in Table 14. The materials were 112 individuals which were caught by gill net and fixed shore net offshore from Takasago and Murotsu, located in the Seto Inland Sea side of Hyogo Prefecture, from July to September, 1993. The specimens were classified into the dotted and non-dotted types by the presence or absence of the black dots on the body. The average total length was 229.4 mm in the dotted type and 253.4 mm in the non-dotted type, respectively, and all of the individuals were regarded as 1-year-old fish (Ochiai and Tanaka, 1986) which were born in 1992. The obtained specimens were morphologically and genetically examined by the common methods

Fig. 27. General aspects of dotted type of ***Lateolabrax japonicus***.

Table 14. Collecting and treating data of examined specimens

Type	Dotted	Non-dotted
Period of collection	Jul.,1993－Sept.,1993	Jul.,1993－Sept.,1993
Locality	Takasago, Murotsu (Hyogo)	Takasago, Murotsu (Hyogo)
Method of sampling	Gill net, Fixed shore net	Gill net, Fixed shore net
Number of individuals	26	86
Range of size (TL,mm)	181.1－278.3	237.2－276.6
Average size (TL,mm)	229.4	253.2
Preservation	Frozen in -30℃ before electrophoresis, thereafter fixed by 10% formalin	Frozen in -30℃ before electrophoresis, thereafter fixed by 10% formalin

noted in section 2. As the genetic markers, 20 isozymic loci were used, the as same as in sections 3－5.

Characteristics of dotted and non-dotted types

Initially, isozymes were detected for the specimens and the ***PROT-1**** locus in which alleles are replaced between ***L. japonicus*** and ***L.*** sp. (Fig. 12) was examined in order to identify the species. The ***PROT-1**** of all the individuals except one showed homozygotes with the ****100*** allele, corresponding with ***L. japonicus***.

However, one individual of the dotted type whose No. is K-18 possessed a heterozygote with the ****100*** allele of ***L. japonicus*** and the ****170*** allele of ***L.*** sp., so it was thought that this individual may be an F1 hybrid between ***L.*** sp. escaped from culture cages (see section 4) and native ***L. japonicus***.

Table 15 shows the allelic frequencies at polymorphic loci of ***L. japonicus***, ***L.*** sp. and K-18, together with probable genotypes of K-18 when an F1 hybrid between ***L. japonicus*** and ***L.*** sp. occurs, which are calculated from the allelic frequencies of the two species.

At the ***PROT-1**** locus, the probability that the hybrid will be heterozygous is 100% because alleles are completely replaced between ***L. japonicus*** and ***L.*** sp. (Fig. 12). At the many other loci, there is reasonable probability to suppose that K-18 is an F1 hybrid. However at the ***LDH-2**** locus, the fact that K-18 possessed a homozygote with the ****-100*** allele, which is the main allele of ***L. japonicus,*** reduced the F1 probability to a low value of 3.1% (Table 15).

For the morphological characters of K-18, the number of pored lateral line scales (LLS), the number of total gill rakers (GR) and the number of vertebrae (VT), in which significant differences were recognized between ***L. japonicus*** and ***L.*** sp. (Figs. 6, 7, 9), were 80, 27 and 36, respectively, corresponding well with the ranges of ***L. japonicus***.

The average proportion values of length-measured characters of the dotted and non-dotted types, together with results of ***t*** tests between both types, and those of

Table 15. Probability that K-18 is F_1 hybrid between ***L. japonicus*** and ***L*** . sp.

Locus	Allele		Frequency		Genotype of K-18	Probability for F_1
			L . ***japonicus*** [1]	***L*** . sp.[1]		
AAT-1*	****100***	A	0.792	0.981	AA	0.777
	****85***	B	0.208	0.019		
AAT-2*	****-100***	A	1.000	1.000	AA	1.000
ADH*	****-50***	C	0.011	0.000	AA	0.660
	****-100***	A	0.660	1.000		
	****-150***	B	0.330	0.000		
CK*	****100***	A	1.000	1.000	AA	1.000
G3PDH-1*	****100***	A	1.000	1.000	AA	1.000
G3PDH-2*	****100***	A	0.986	1.000	AA	0.986
	****-200***	B	0.014	0.000		
GPI-1*	****130***	C	0.000	0.060	AB	0.832
	****110***	B	0.100	0.922		
	****100***	A	0.900	0.017		
GPI-2*	****-100***	A	0.938	0.940	AA	0.881
	****-250***	B	0.063	0.060		
IDDH*	****165***	C	0.037	0.200	AA	0.770
	****100***	A	0.963	0.800		
IDHP-1*	****100***	A	0.912	0.588	AA	0.536
	****70***	B	0.088	0.412		
LDH-2*	****100***	B	0.097	0.966	AA	0.031
	****-100***	A	0.903	0.034		
MDH-1*	****100***	A	0.986	1.000	AA	0.986
	****70***	B	0.014	0.000		
MDH-2*	****-100***	A	1.000	1.000	AA	1.000
MEP*	****150***	C	0.079	0.050	AA	0.797
	****100***	A	0.886	0.900		
	****50***	B	0.035	0.050		
MPI-1*	****125***	B	0.306	0.363	AA	0.442
	****100***	A	0.694	0.638		
MPI-2*	****100***	A	1.000	0.987	AA	0.987
	****75***	B	0.000	0.013		
PGDH*	****120***	C	0.000	0.011	AA	0.989
	****100***	A	1.000	0.989		
PGM*	****115***	D	0.021	0.060	AA	0.233
	****100***	A	0.465	0.500		
	****75***	B	0.507	0.431		
	****55***	C	0.007	0.009		
PROT-1*	****170***	B	0.000	1.000	AB	1.000
	****100***	A	1.000	0.000		
SOD-1*	****100***	A	1.000	0.964	AA	0.964
	****20***	B	0.000	0.036		

[1] Data from Table 5

L. japonicus and ***L***. sp. to be referred to are shown in Table 16. Further, the average values of the dotted and non-dotted types and those of ***L. japonicus*** and ***L***. sp. were compared and the relationships of the value sizes were expressed by unequal signs, giving circle signs at the right edge of the table for the characters in which the directions of the unequal signs of the two relationships correspond to each

Table 16. Length-measured characters of dotted and non-dotted sea bass with t values between both types, and those of ***L. japonicus*** and ***L.*** sp. to be referred

Type or Species	Average value			t		Average value			Correspondence
	Dotted		Non-dotted			***L. japonicus*** [1]		***L.*** sp.[1]	
Total length[2]	122.08	>	121.52	0.904		121.69	>	119.17	○
Fork length[2]	115.98	>	115.75	0.633		115.11	>	114.00	○
Pre-anus length[2]	66.42	>	65.65	1.333		66.04	<	66.76	
Body depth[3]	25.44	<	25.61	0.500		24.24	<	26.28	○
Body width[2]	14.41	>	13.77	1.599		13.38	<	13.46	
Caudal peduncle depth[2]	10.02	>	9.79	2.031		9.28	<	10.35	
Caudal peduncle length[2]	21.64	<	21.87	0.779		21.61	<	22.09	○
Pre-dorsal length[2]	35.38	<	35.57	0.434		35.24	>	35.00	
First dorsal fin length[2]	14.74	>	13.98	2.181	*	14.10	>	12.62	○
Second dorsal fin length[2]	11.41	<	12.64	3.383	**	11.82	<	12.57	○
Anal fin length[2]	13.21	>	13.02	0.692		12.49	<	14.33	
Pectoral fin length[2]	18.06	>	17.12	3.144	**	17.05	>	16.08	○
Pelvic fin length[2]	19.08	>	18.25	3.459	**	17.57	<	18.33	
Head length[2]	32.56	>	31.90	1.767		31.98	<	32.56	
Snout length[3]	25.27	<	25.94	1.786		26.25	>	25.10	
Orbital diameter[3]	18.50	<	20.15	4.023	***	17.70	<	24.83	○
Interorbital width[3]	20.15	<	20.32	0.426		21.20	<	21.64	○
Suborbital width[3]	8.53	<	8.81	0.634		11.26	<	10.72	○
Upper jaw length[3]	42.97	<	43.05	0.200		42.36	<	44.21	○
Lower jaw length[3]	46.53	<	46.92	0.897		46.43	<	46.82	○
Pectoral scaly area length[4]	26.05	>	25.33	0.738		26.73	>	19.43	○

[1] Data from Table 3
[2] Percentage of standard length
[3] Percentage of head length
[4] Percentage of pectoral fin length
* Significant at 5% level
** Significant at 1% level
*** Significant at 0.1% level

Table 17. Meristic characters of dotted and non-dotted sea bass with t values between both types, and those of ***L. japonicus*** and ***L.*** sp. to be referred

Type or Species	Average value			t		Average value			Correspondence
	Dotted		Non-dotted			***L. japonicus*** [1]		***L.*** sp.[1]	
Dorsal fin spines	12.91	>	12.88	0.171		12.85	<	12.95	
Dorsal fin soft rays	12.64	<	13.38	3.210	**	12.78	<	13.06	○
Anal fin spines	3.00	=	3.00	0.000		3.00	>	2.98	
Anal fin soft rays	7.27	<	7.65	1.805		7.73	>	7.53	
Pectoral fin soft rays	17.18	>	16.92	1.159		16.85	>	16.31	○
Pelvic fin spines	1.00	=	1.00	0.000		1.00	=	1.00	○
Pelvic fin soft rays	5.00	=	5.00	0.000		5.00	=	5.00	○
Pored scales on lateral line	84.64	>	81.58	2.845	**	83.08	>	72.85	○
Scales above lateral line	16.09	>	15.31	0.454		14.50	>	15.34	○
Scales below lateral line	19.09	>	18.50	0.335		17.98	>	17.92	○
Gill rakers (upper limb)	8.46	>	7.96	1.541		9.66	>	6.38	○
Gill rakers (lower limb)	18.27	>	17.85	1.881		17.52	>	14.70	○
Gill rakers (total)	26.73	>	25.81	2.065	*	27.18	>	21.07	○
Vertebrae	35.91	>	35.73	0.846		35.89	>	34.95	○

[1] Data from Table 3
* Significant at 5% level
** Significant at 1% level

other.

The results of ***t*** tests between the dotted and non-dotted types showed significant differences in the first and second dorsal fin lengths, pectoral and pelvic fin lengths and orbital diameter, indicating that the two types differed in particular in the length of the fins.

The relationships of the proportion sizes between the dotted and non-dotted types and those of ***L. japonicus*** and ***L.*** sp. corresponded to each other in many of the characters, in particular the characters in the head, except the snout length, completely corresponded to each other (Table 16). Also, in the characters in which significant differences were recognized between the average values of ***L. japonicus*** and ***L.*** sp., the two relationships completely corresponded to each other except for the pelvic fin length (Table 16).

For the meristic characters, the average values of the dotted and non-dotted types together with results of ***t*** tests between both the types, and those of ***L. japonicus*** and ***L.*** sp. to be referred to are shown in Table 17. Further, the average values of the dotted and non-dotted types and those of ***L. japonicus*** and ***L.*** sp. were compared and relationships of the value sizes were expressed by signs of inequality or equality, giving circle signs at the right edge of the table for the characters in which the directions of the unequal or equal signs of the two relationships correspond to each other.

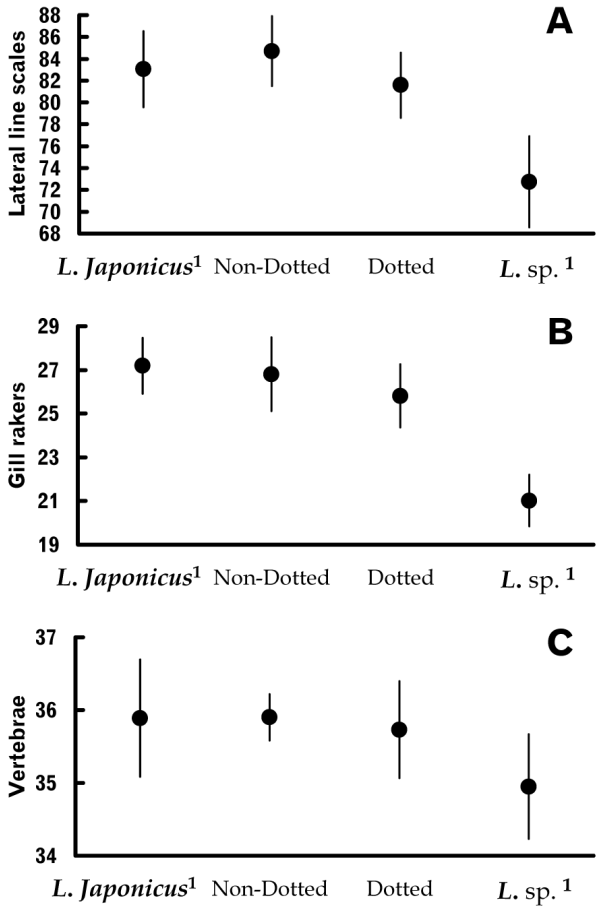

Fig. 28. Graphic comparisons of significant meristic characters in dotted and non-dotted types, together with data for ***Lateolabrax japonicus*** and ***L.*** sp. [1] Data based on section 3. Dark circles indicate average values of each lot. Longitudinal bars indicate standard deviations (unbiased value). A: Number of pored lateral line scales (LLS); B: Number of total gill rakers (GR); C: Number of vertebrae (VT).

The results of ***t*** tests between the average values of the dotted and non-dotted types showed significant differences in the number of dorsal fin spines, the number of pored lateral line scales, and the number of total gill rakers, indicating that the two types differed also meristically (Table 17). The relationships of average value sizes between the dotted and non-dotted types and those of ***L. japonicus***

Table 18. Allelic frequencies of dotted and non-dotted sea bass with χ^2 heterogeneities between both types, and those of *L. japonicus* and *L.* sp. to be referred

Locus	Allele	Allelic frequency		χ^2 hetero	Allelic frequency	
		Non-dotted	Dotted		*L. japonicus*	*L.* sp.[1]
*AAT-1**	***120**	0.024	0.000	1.156	0.000	0.000
	***100**	0.833	0.875	0.315	0.792	0.981
	***85**	0.143	0.125	0.062	0.208	0.019
*AAT-2**	***-50**	0.000	0.019	2.668	0.000	0.000
	***-100**	1.000	0.981	2.668	1.000	1.000
*ADH**	***-50**	0.006	0.000	0.311	0.011	0.000
	***-100**	0.690	0.654	0.246	0.659	1.000
	***-150**	0.304	0.346	0.334	0.330	0.000
*CK**	***100**	1.000	1.000	—	1.000	1.000
*G3PDH-1**	***100**	1.000	1.000	—	1.000	1.000
*G3PDH-2**	***100**	0.935	1.000	3.540	0.986	1.000
	***50**	0.065	0.000	3.540	0.014	0.000
*GPI-1**	***130**	0.000	0.000	—	0.000	0.060
	***110**	0.088	0.173	2.402	0.100	0.922
	***100**	0.912	0.827	2.402	0.900	0.018
*GPI-2**	***-100**	0.968	1.000	1.694	0.937	0.940
	***-250**	0.032	0.000	1.694	0.063	0.060
*IDDH**	***165**	0.120	0.120	0.000	0.037	0.200
	***100**	0.880	0.860	0.134	0.963	0.800
	***-50**	0.000	0.020	3.335	0.000	0.000
*IDHP-1**	***120**	0.006	0.000	0.292	0.000	0.000
	***100**	0.965	1.000	1.793	0.912	0.588
	***70**	0.029	0.000	1.487	0.088	0.412
*LDH-2**	***100**	0.060	0.192	8.288 **	0.097	0.966
	***-100**	0.940	0.808	8.288 **	0.903	0.034
*MDH-1**	***100**	0.933	1.000	3.675	0.986	1.000
	***70**	0.067	0.000	3.675	0.014	0.000
*MDH-2**	***-100**	1.000	1.000	—	1.000	1.000
*MEP**	***150**	0.045	0.115	2.410	0.079	0.050
	***100**	0.898	0.885	0.059	0.886	0.900
	***50**	0.057	0.000	3.064	0.035	0.050
*MPI-1**	***125**	0.275	0.375	1.765	0.306	0.363
	***100**	0.725	0.625	1.765	0.694	0.638
*MPI-2**	***100**	1.000	1.000	—	1.000	0.987
	***75**	0.000	0.000	—	0.000	0.013
*PGDH**	***120**	0.000	0.000	—	0.000	0.011
	***100**	0.994	1.000	0.304	1.000	0.989
	***75**	0.006	0.000	0.304	0.000	0.000
*PGM**	***115**	0.006	0.000	0.315	0.021	0.060
	***100**	0.464	0.500	0.208	0.465	0.500
	***75**	0.530	0.500	0.144	0.507	0.431
	***55**	0.000	0.000	—	0.007	0.009
*PROT-1**	***170**	0.000	0.019	0.429	0.000	1.000
	***100**	1.000	0.981	0.429	1.000	0.000
*SOD-1**	***145**	0.045	0.019	0.404	0.000	0.000
	***100**	0.955	0.981	0.404	1.000	0.964
	***20**	0.000	0.000	—	0.000	0.036

[1] Data from Table 5 ** Significant at 1% level

and ***L***. sp. corresponded to each other in most of the meristic characters, in particular those relationships completely corresponded to each other in the characters in which significant differences were recognized (Table 17).

Graphic comparisons of significant meristic characters in dotted and non-dotted types, together with data for ***L. japonicus*** and ***L***. sp., are shown in Fig 28. It can be visually understood that the dotted type inclined towards ***L***. sp. in all of these key characters.

For the genetic characteristics, allelic frequencies of the dotted and non-dotted types with χ^2 heterogeneities between the two types, together with those of ***L. japonicus*** and ***L***. sp. to be referred to are shown in Table 18.

The χ^2 test showed high significance at the ***LDH-2**** locus and the χ^2 values at the ***GPI-1**** locus were close to the significant level (Table 18). At the two loci, the alleles were replaced almost completely between ***L. japonicus*** and ***L***. sp. (Table 5).

Pie graph comparisons of allelic frequencies at significant loci, including the ***LDH-2**** and ***GPI-1**** in the dotted and non-dotted types, together with data for ***L. japonicus*** and ***L***. sp., are shown in Fig. 29. Allelic compositions of the dotted and non-dotted types differed from each other considerably, the compositions of the dotted type being closer to those of ***L***. sp. rather than ***L. japonicus***.

The genetic distance (D values) (Nei, 1972) between the dotted type and ***L***. sp.,

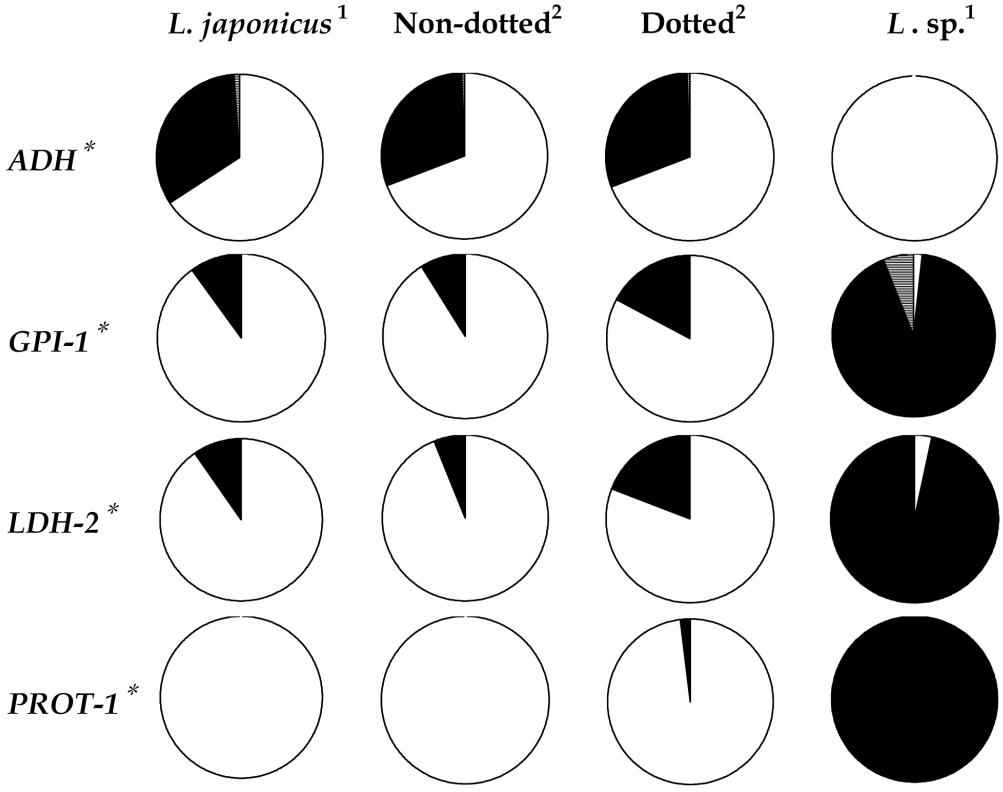

Fig. 29. Pie graph comparisons of allelic frequencies at significant loci in dotted and non-dotted types, together with data for ***Lateolabrax japonicus*** and ***L***. sp. [1] Data from Table 5; 2 Data from Table 18.

and the non-dotted type and *L*. sp. resulted in 0.155 and 0.180, respectively, showing that the dotted type is genetically closer to *L*. sp. than the non-dotted type.

Characteristics of dotted and non-dotted types in the Ariake form

Graphic comparisons of significant meristic characters in dotted and non-dotted types in the Ariake form (hereafter called Ariake dotted type and Ariake non-dotted type, respectively), together with data for *L. japonicus* and *L*. sp., are shown in Fig 30. The Ariake dotted type inclined towards *L*. sp. in LLS and VT.

Pie graph comparisons of allelic frequencies at significant loci in the Ariake dotted and Ariake non-dotted types, together with data for *L. japonicus* and *L*. sp., are shown in Fig. 31. Allelic compositions of the Ariake dotted and Ariake non-dotted types differed from each other considerably, the compositions of the Ariake dotted type being closer to those of *L*. sp. rather than *L. japonicus*.

The genetic distance (D values) (Nei, 1972) between the Ariake dotted type and *L*. sp., and the Ariake non- dotted type and *L*. sp. resulted in 0.106 and 0.135, respectively, showing that the Ariake dotted type is genetically closer to *L*. sp. than the Ariake non-dotted type.

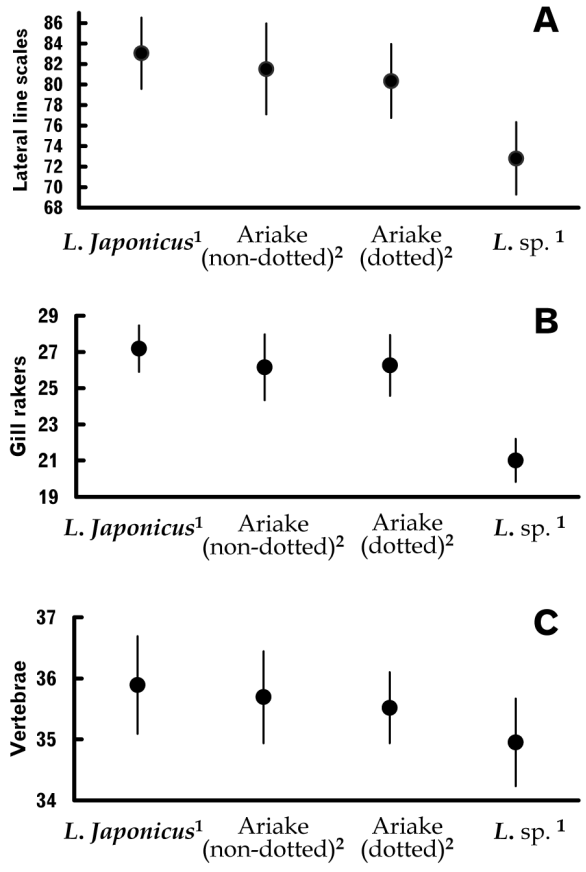

Fig. 30. Graphic comparisons of significant meristic characters in Ariake dotted and Ariake non-dotted types, together with data for ***Lateolabrax japonicus*** and *L*. sp. [1] Data based on section 3. [2] Data based on section 5. Dark circles indicate average values of each lot. Longitudinal bars indicate standard deviations (unbiased value). A: Number of pored lateral line scales (LLS); B: Number of total gill rakers (GR); C: Number of vertebrae (VT).

The morphological and the genetic relationships between the Ariake dotted and Ariake non-dotted types were very similar to those between the dotted and the non-dotted types in *L. japonicus*.

Discussion

For the dotted and non-dotted types of *L. japonicus*, because one individual of the dotted type possessed a heterozygote with the ****100*** and ****170*** alleles at the ***PROT-1**** locus, the possibility that the individual was an F1 hybrid between *L. japonicus* and *L*. sp. was considered. However, such a possibility is very low because the individual possessed a homo-

zygote with the *-**100** allele, which is the main allele of ***L. japonicus,*** at the ***LDH-2**** locus (Table 15).

Therefore, there can be other possibilities for the origin of the individual—i.e., that it is in a subsequent F2 generation from the hybrid, or that the ***170** allele which is peculiar to ***L.*** sp. is included in the ***L. japonicus*** population. However, the former can be rejected because the facts that the import of ***L.*** sp. began in 1990 (Matsuoka, 1993) and the maturative age of ***L.*** sp. is over 3 years old (Wu et al., 1979; Liu and Qin, 1987) do not suggest the possibility that ***L.*** sp. had inherited more than 2 generations in 1993 when the individual was obtained. Consequently, the origin of the individual is strongly suggested to be the latter possibility. Actually, it will be reported in section 7 that some of the ***L. japonicus*** populations in Japan and Korea include the ***170** allele at the ***PROT-1**** locus, albeit infrequently (Table 21).

The relationships in the morphological and genetic characteristics of the dotted and non-dotted types and those of ***L. japonicus*** and ***L.*** sp. corresponded to each other in many of the characters, and the dotted type was morphologically and genetically closer to ***L.*** sp. rather than ***L. japonicus***. A very similar fact was observed in the relationship between the Ariake dotted and Ariake non-dotted types. Thus, the dotted type is morphologically closer to ***L.*** sp. because it is genetically closer to ***L.*** sp.—i.e. the black dots which are peculiar to ***L.*** sp. can appear. This may be a

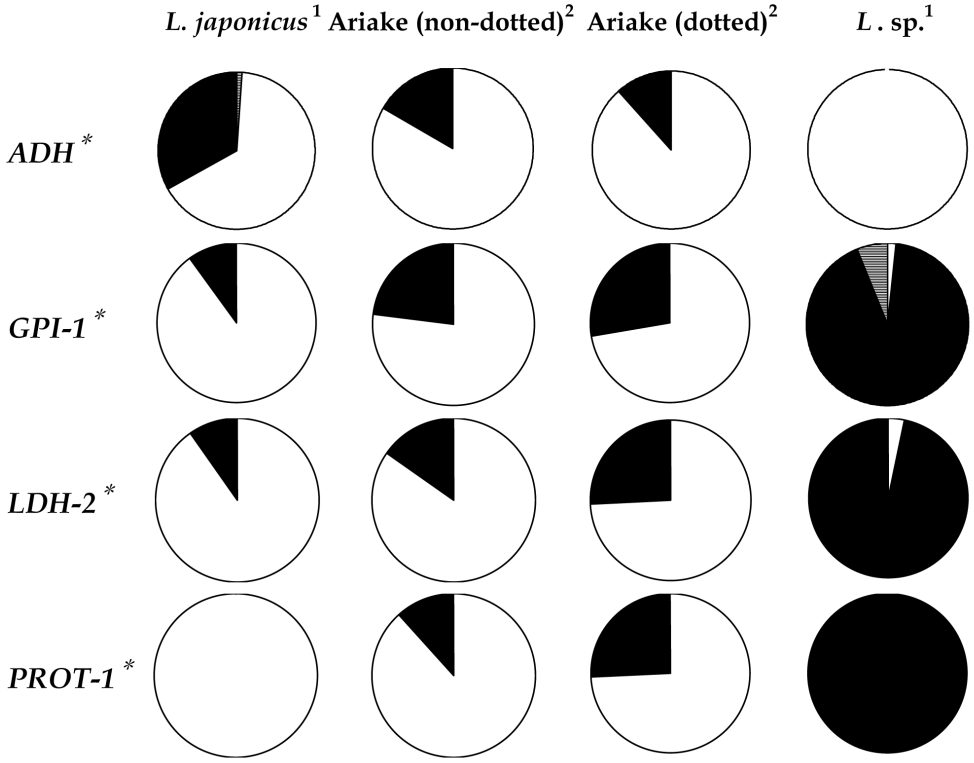

Fig. 31. Pie graph comparisons of allelic frequencies at significant loci in Ariake dotted and Ariake non-dotted types, together with data for ***Lateolabrax japonicus*** and ***L.*** sp. [1] Data from Table 5; [2] Data from Table 13.

mechanism common to the dotted type of ***L. japonicus*** and the Ariake dotted type.

The characters of living beings are ruled by many alleles, and in ***L. japonicus*** and ***L.*** sp. there can be many alleles which are common and peculiar to each species. Because the two species are highly similar to each other, even to the point of being treated as a uniform species (Katayama, 1960a, 1960b), individually some of them can possess alleles peculiar to another species. Therefore, in the ***L. japonicus*** population, the black dots appear in individuals which possess many alleles peculiar to ***L.*** sp. quantitatively, and also such individuals are morphologically closer to ***L.*** sp. Thus, the appearance of dots might be regarded as the effect of polygenes, and the fact that the number and shape of dots vary considerably also suggests such a possibility.

Here, several hypotheses for a process by which the dotted type has appeared in the ***L. japonicus*** population, except the Ariake form, are considered as follows:

Hypothesis 1: As ***L.*** sp. has been introduced into Japan artificially and affected the native populations genetically, the alleles peculiar to ***L.*** sp. have been included in the ***L. japonicus*** population.

Hypothesis 2: During the divergence of ***L. japonicus*** and ***L.*** sp. from a common ancestral species, the alleles peculiar to ***L.*** sp. inherited from the ancestor remain in the ***L. japonicus*** population.

Hypothesis 3: Following the divergence of ***L. japonicus*** and ***L.*** sp., the latter naturally introgressed into the ***L. japonicus*** population genetically, resulting in the continuing existence of the alleles peculiar to ***L.*** sp. in the ***L. japonicus*** population.

In regard to hypothesis 1, the import of ***L.*** sp. began in 1990 (Matsuoka, 1993), although there is no conclusive evidence that another import had not been made before that time, there is hardly any possibility that long-term massive imports could have genetically affected the ***L. japonicus*** population.

As for hypothesis 2, Nei (1990) explained a process by which alleles are completely replaced at certain loci on the basis of probability, stating that allelic frequency changes gradually with generations and are finally fixed by the other alleles.

According to this theory, during the process of the alleles (which are responsible for the dot- or no dot-appearance) proceeding to complete replacement, the alleles of another species can be included in both the ***L. japonicus*** and ***L.*** sp. populations to a small degree of frequency. Therefore, in the ***L. japonicus*** population, the dots appear in individuals which possess the dot- allele of ***L.*** sp., but on the other hand, the presence of a few individuals which lack the dots in the ***L.*** sp. population (Fig. 10) suggests that the dots do not appear in ***L.*** sp. individuals with the non-dot-allele of ***L. japonicus***.

For hypothesis 3, two possibilities are considered. That is, the number of years of divergence between ***L. japonicus*** and ***L.*** sp. is calculated from the genetic distance as being about 0.9 Ma (Nei, 1990), and thereafter it is hypothesized that the Continent and the Japanese archipelago were sometimes connected or close to each other due to diastrophism and climate changes (Kaseno, 1975, 1994; Oba, 1993). During this time, ***L. japonicus*** and ***L.*** sp., which had just diverged, could hybridize in a broad area which was the border between the Continent and the Japanese archipelago. Thereafter, such hybridized populations might have been genetically assimilated into the mass populations of ***L. japonicus*** or ***L.*** sp.

However, the alleles of another species which introgressed at this time have not been rejected completely from each population, so they may be preserved in each population to a small degree of frequency. Further, the sea bass from the Ariake Sea, which is genetically strongly influenced by ***L.*** sp. (see section 5), can be regarded as a geographically isolated population originating from the hybrids which occurred by this event.

Another possibility in hypothesis 3 is that a little gene flow occurs constantly, even presently. In the case of ***L.*** sp., since the pelagic period of its eggs and larvae can be rather long, the same as that of ***L. japonicus*** (Wan and Chen, 1988), the larvae and juveniles of ***L.*** sp. which occurred around the Continental coast can wash to the coast of the Japanese archipelago and mature there and they might genetically influence the ***L. japonicus*** population.

However, the two species are now experiencing reproductive isolation ecologically, because there is no genetic interchange at the southwestern edge of the Korean Peninsula, which is the border area of distribution of ***L. japonicus*** and ***L.*** sp. (see section 7), so this possibility is rejected. Therefore, according to hypothesis 3, the dotted type appears because the genetic influence of ***L.*** sp. on ***L. japonicus*** still remains presently.

Acknowledgments

The author heartily thanks Dr. Nobuo Shimamoto, Hyogo Prefectural Fisheries Experimental Station for willingly providing many sea bass specimens. Dr. Nobuhiko Taniguchi, Faculty of Agriculture, Kochi University (present address: Tohoku University), made available equipment for electrophoretic analyses and gave much support and advice. Finally, I wish to thank Mr. Roger Ahlberg, Japan English Service, Inc., Chiba, for checking the manuscript.

7 Genetic variations in local populations of *Lateolabrax japonicus* and *L.* sp. around east Asian coasts

The studies carried out in the earlier sections revealed that the Japanese and Chinese sea bass are distinct species and that a hybridized population of them exists in the Ariake Sea. Further, the mechanism by which the black dots appear was explained by the theory of gene flow following introgressive hybridization.

In this section, genetic variations in local populations of ***Lateolabrax japonicus*** and ***L.*** sp. around east Asian coasts including Japan, Korea, Taiwan and China were examined by use of isozymes and mitochondrial DNA (hereafter called mt-DNA) as genetic markers, in order to clarify the population structures and distributive status of the two species.

Materials and methods

Data on the collection of specimens examined in this section are shown in Table 19, and the collection localities are illustrated in Fig. 32. Samples were obtained from 20 localities around the Japanese archipelago, the Korean Peninsula, Taiwan and the Chinese coast. For isozyme analysis, specimens from Fuzhou and Xiamen in China were combined as Fujian sample because individual numbers from the two localities were somewhat insufficient for calculation. Therefore it turned out to be 19 specimens for isozyme analysis.

For mt-DNA analysis, the Fuzhou and the Xiamen samples were separately treated, and the Taiwan sample was not available for analysis because DNA extraction from muscle tissues was not successful due to a problem of lengthy preservation in ethanol. Further, the Yentai sample was not examined. Consequently, it turned out to be 18 samples for mt-DNA analysis.

Isozymes were examined by the common methods noted in section 2. As genetic markers, 28 loci which are shown in Table 1, except the ***SOD-2****, were used; however, some loci were not examined for some samples from China and Taiwan. Genetic distances (D values) between the samples were calculated from isozymic allele frequencies after Nei (1972), and a dendrogram of genetic relationships between the samples was constructed from the genetic distances by the UPGMA method.

For the mt-DNA analysis, samples from muscle or fin clips were stored in 95% ethanol or a refrigerator for DNA extraction. Genomic DNA was prepared from the samples following Xiao et al. (2001). Fish primers L16550 (5'-TCACCCCTGG CTCCCAAAGCCAG-3') and H455 (5'-TGCATATAAAAGAATGCCGGCATG-3'), which target a portion of transfer RNA (t-RNA)-pro and the central conserved re-

Table 19. Collection data of specimens examined

Example name	Date of collection	Original locality	Method of sampling	Individuals[1]	Remarks
Tokyo	24-29 Jan. 1995	off Futtsu, Chiba Pref., Japan	Trawling	48 (10)	
Mikawa	25 Aug. 1995	Mikawa bay, Aichi Pref., Japan	Boat seine	43 (10)	
Harima	Dec., 1995 and Dec., 1996	off Hiketa, Kagawa Pref., Japan	Trawling	37 (10	
Suo	6-19 Dec. 1993	off Aio, Yamaguchi Pref., Japan	Fixed shore net	32 (10)	
Yatsushiro	14 Sep. 1995	Kuma River estuary, Kumamoto Pref.,	Angling	35 (25)	
Ariake	18-26 May, 1993	off Shimabara, Nagasaki Pref., Japan	Angling	40 (14)	
Omura	13 Oct. 1999	Omura Bay, Nagasaki Pref., Japan	Angling	21 (10)	
Tottori	17-25 Sep. 1994	Tottori, Tottori Pref., Japan	Angling	39 (10)	
Maizuru	14 May, 2000	Maizuru Bay, Kyoto Pref., Japan	Fixed shore net	35 (10)	
Ishikawa	22 Aug. -2 Sep. 1995	Kanazawa, Ishikawa Pref., Japan	Angling	82 (10)	
Hadong	Feb., 1995	A river in Hadong, Kyeonsangnamdo, Korea		30 (21)	Caught in Hadong in summer of 1994, thereafter cultured in a nursery in Koje Island, Korea
Yeosu	14 Oct., 2002	Tolsan Island, Yeosu, Cheollanamdo, Korea		40 (9)	Caught around Tolsan Island, thereafter cultured in a nursery there
Mokpo		Mokpo, Cheollamamdo,		34 (8)	
Yentai	11 Nov., 1995	Yentai, Shandong Prov., China		40	Caught in Yentai, thereafter transported into Japan and cultured in a nursery in Hiketa
Taiwan	May, 1994	Fang Yuan, Changhua County, Taiwan		52	Fingerlings produced from artificially fertilized eggs imported from Taiwan
Weihai	Nov., 2000	Weihai, Shandong Prov., China		50 (22)	
Zhoushan	Nov., 2000	Zhoushan Islands, Zhejian Prov., China		27 (10)	
Fuzhou	Nov., 1999	Fuzhou, Fujian Prov., China		10 (10)	For isozyme analysis, specimens from Fuzhou and Xiamen were combined as Fujian
Xiamen	Nov., 1999	Xiamen, Fujian Prov., China		19 (16)	For isozyme analysis, specimens from Fuzhou and Xiamen were combined as Fujian
Beihai	Oct., 1999	Weihai, Guangdong Prov., China		40 (25)	

[1] Numbers in upper side with no parentheses indicate individuals examined for isozyme, while those in lower parentheses indicate individuals examined for mt-DNA

gion of the mitochondrial control region, were used for the polymerase chain reac tion (PCR) to amplify the first hypervariable region in a 50-μl reaction. PCR mixtures contained 50 ng DNA, 10 pmol primers, 200 μM dNTPs, 1×PCR Buffer (SABC), and 2 units of Taq DNA polymerase (SABC). The reaction was performed in a Biometra thermocycler as follows: denaturation at 95℃ for 5 minutes and 40 cycles of denaturation at 95℃ for 45 seconds, annealing at 56℃ for 45 seconds and extension at 72℃ for 45 seconds, followed by a final extension for 10 minutes at 72 ℃. All sets of PCR included a negative control reaction tube in which all reagents were included, with the exception of template DNA. PCR products purified on spin columns (Watson BioTechnologies Inc., Shanghai) were directly sequenced for both strands using a Big-Dye Sequencing Kit (Applied Biosystems). The sequencing primers were the same as those used in PCR amplification.

The sequences were edited and aligned using Dnastar software (DNASTAR, Inc.), and the sequence haplotypes were identified. A neighbor-joining (Saitou and Nei, 1987) tree of haplotypes was computed with a two-parameter model of sequence evolution after Kimura (1980). MEGA2 (Kumar et al., 2001) was used to construct the neighbor-joining tree. The evolutionary relationships among haplotypes were inferred from a minimum-spanning tree constructed with ARLEQUIN version 2.0 (Schneider et al., 2000). That method used a parsimony approach to connect each sequence to its closest neighbor, based on pairwise differences, and differed from traditional methods of tree construction by allowing extant haplotypes to occupy internal nodes.

Inter and intra-population variability were computed with ARLEQUIN, and MEGA2. Variation within populations was expressed as haplotype diversity (H),

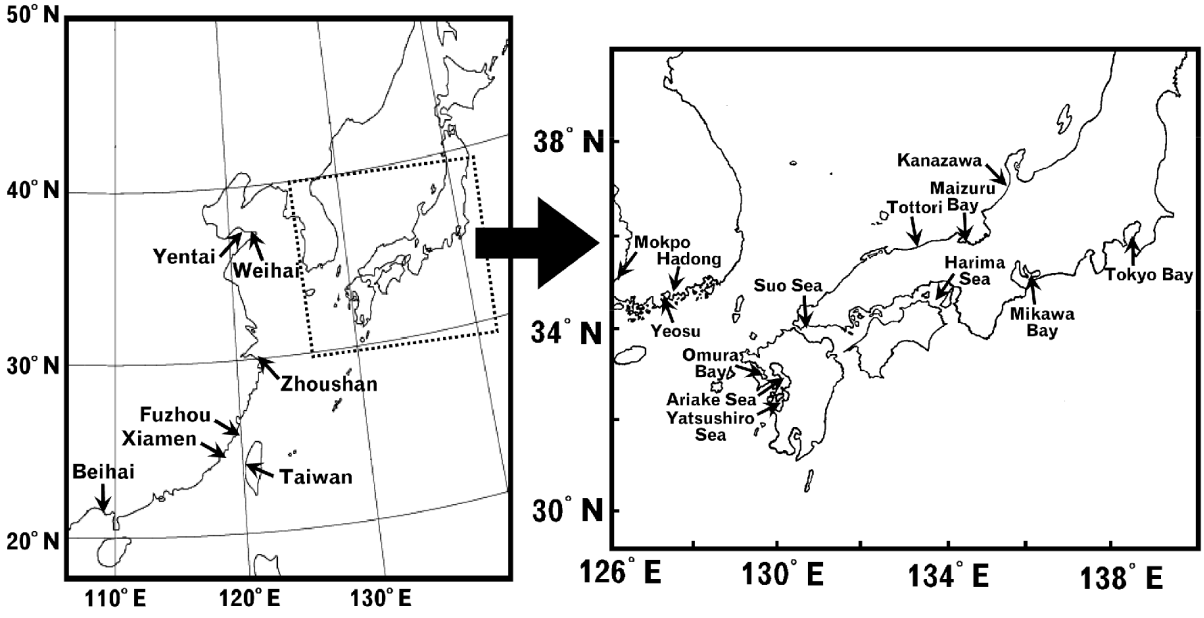

Fig. 32. Collection localities of specimens examined.

nucleotide diversity (л), number of segregating sites (S), and average number of pairwise difference (dx) among haplotypes, assuming the two-parameter model of sequence evolution (Kimura, 1980). The population structure was evaluated using the molecular variance software package (AMOVA) in ARLEQUIN. Statistical confidence in variance estimates was determined by comparing the observed Φ statistics against a distribution of estimates generated from 10,000 permutations of data (Excoffer et al., 1992).

Estimates of female-mediated gene flow (N) were calculated from the pairwise values of FST. The values of FST are equivalent to ΦST (Hudson et al. 1992) and can therefore be used to estimate Nemf in the equation Nemf =((1/ FST) 1)/2 (Weir and Cockerham, 1984). The estimation of gene flow assumes that populations are at genetic equilibrium. Genetic equilibrium results when a balance between migration and genetic drift is achieved, such that the distribution of haplotypes within populations connected by gene flow is a result of a recent process rather than historical associations. Under a stepping-stone model, when genetic equilibrium is achieved, a pattern of distance isolation is observed and the gene flow between populations decreases with increasing geographical distance. Log Nemf was plotted against the log of the geographical distance (K) between localities to examine isolation by distance (Slatkin, 1993). The statistical confidence in the relationship between log Nemf and log K was determined using the Mantel test statistic from IBD Version 1.4 (Bohonak, 2002).

The demographic history of each population was examined by two different approaches. First, the Fs test of Fu (1997) was used to test if neutrality holds (i.e., the population under study evolves with a constant effective population size, all mutations being selectively neutral). A population that has experienced population expansion may result in a rejection of null hypothesis. Historical demographic expansions were also detected by examination of frequency distributions of pairwise differences of sequences (mismatch distribution) within populations or regional assemblages (Rogers and Harpending, 1992).

Mismatch distribution analysis was used to evaluate whether there was a signature of population expansion that was proposed for marine fish populations in the mismatch, and the timing of demographic expansion was measured in units of mutational time. Typically, a population with a constant size in the past has a multimodal mismatch distribution, while a population that has undergone expansion usually shows a unimodal or poisson-like distribution (Rogers and Harpending, 1992; Rogers, 1995).

Concordance of the obtained data with the distribution underlying the sudden-expansion model of Rogers (1995) was assessed by means of a least-squares ap-

proach (Schneider and Excoffer, 1999) implemented by ARLEQUIN.

For distribution that did not differ significantly (P>0.05) from the expectation of the sudden expansion model, τ values (an estimate of the mode of the mismatch distribution) were estimated, which are indices of time since expansion is expressed in units of mutational time (Rogers and Harpending, 1992). Thevalues of τ were transformed to estimates of real time since expansion is calculated with the equation $\tau = 2ut$, where u is the mutation rate for the sequence under study and t is the time measured in years since expansion.

The same estimates of the mutation rate for the mitochondrial control region described previously were used for divergence estimates. Because small sample sizes could contributed to bumpy mismatch distributions (Yao et al., 2002) and sample sizes of most of the examined ***L. japonicus*** specimens were small, being no larger than 10, the mismatch distribution analysis and Fs test of neutrality were introduced for regional groups of populations defined in the AMOVA analysis.

Results of isozyme

Initially, the fitness of the allelic frequencies in polymorphic loci, according to the Hardy-Weinberg equilibrium, was examined by chi-square tests in all lots of the local samples. In all of the samples, except those of Mokpo in Korea and Taiwan, no χ^2 values were shown to have been significant at the 5% level, therefore they were regarded as representing simple Mendelian populations but in the Mokpo sample, significance was observed at the ***FBALD-1****, ***GPI-1**** and ***LDH-2**** loci. Although most individuals from Mokpo showed the typical genetic features of ***L***. sp., the facts might suggest that the Mokpo samples are a mixture of different populations. Therefore, individual genotypes of all the specimens at the significant loci are shown in Table 20.

Regarding the ***ADH**** locus, because that of ***L***. sp. is fixed with the ***-100**** (A) al-

Table 20. Individuals genotypes of Mokpo sample from Korea

Individual No.	MO-1	MO-2[1]	MO-3[1]	MO-4	MO-5	MO-6	MO-7	MO-8	MO-9	MO-10	MO-11	MO-12	MO-13	MO-14	MO-15	MO-16	MO-17[1]
ADH*	AA	AB	BB	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AB
FBALD-1*	AB	AA	BB	AA	AB	AA	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
FBALD-2*	BB	AB	AA	BB	AD	BD	BD	BB	BE	AB	AB	AB	BB	BD	BE	BE	AA
GPI-1*	BB	AA	AA	BB	BB	BB	BB	BB	BB	BB	BB	BB	AB	BC		BB	AA
LDH-2*	BB	AA	AB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	AB	BB	BB	AB
PROT-1*	BB	AB	AB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	AA

Individual No.	MO-18	MO-19	MO-20	MO-21	MO-22	MO-23[1]	MO-24	MO-25	MO-26	MO-27	MO-28	MO-29	MO-30	MO-31	MO-32	MO-33	MO-34
ADH*	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA	AA
FBALD-1*	BB	BB	BB	BB	BB	AB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
FBALD-2*	AA	AB	BB	AB	AB	AE	BB	BB	BB	BC	BE	BB	BB	AB	BB	BB	AE
GPI-1*	BC	BB	BC		BB	AA	BB	BB	BB	AB		BB	BB	AB	BB	BC	BB
LDH-2*	BB	BB	BB	BB	BB	AA	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB
PROT-1*	BB	BB	BB	BB	BB	AB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB	BB

[1] Presumed to be ***Lateolabrax japonicus*** , whereas the remainder were presumed to be ***L*** . sp

Alleles are symbolized as capital letters, the most dominant allele (****100*** or ****-100***) in ***L*** . ***japonicus*** being A. B and C

lele (Table 5), an individual which possesses a genotype other than AA can be presumed to be ***L. japonicus***. At this point, the No. MO-2, MO-3 and MO-17 individuals can be regarded as ***L. japonicus***. This possibility was also suggested by the fact that those individuals possess dominant alleles of ***L. japonicus*** at the ***GPI-1**** and ***LDH-2**** loci (Table 20), at which significant differences in allelic compositions were recognized between ***L. japonicus*** and ***L.*** sp. (Table 5). Further, the No. MO-23 individual also could be regarded as ***L. japonicus*** by the same reason, although it possesses the AA genotype at the ***ADH**** locus (Table 20).

Regarding the ***PROT-1**** locus at which the alleles were completely replaced between the two species (Table 5), the MO-2, MO-3 and MO-23 were heterozygous with the ***100*** (A) allele of ***L. japonicus*** and the ***170*** (B) allele of ***L.*** sp. However, actually, the ***170*** (B) allele appears in ***L. japonicus*** infrequently (see section 6), and the other ***L. japonicus*** samples from Korea include the ***170*** (B) alleles to a small degree of frequency at the ***PROT-1**** locus (Table 21).

In addition, for the morphological characters of MO-2, MO-3, MO-17 and MO-23, the number of pored lateral line scales (LLS) and the number of total gill rakers (GR), in which significant differences were recognized between ***L. japonicus*** and ***L.*** sp. (Figs. 6, 7, 8), corresponded well with the ranges of ***L. japonicus***. From these facts, it appears reasonable to regard those individuals as ***L. japonicus***.

Consequently, those ***L. japonicus*** individuals were removed from the Mokpo samples. The chi-square tests for fitness of the Hardy-Weinberg equilibrium were remade for this sample, and indicated no significance at any loci. This also suggested that the original sample was a mixture of ***L. japonicus*** and ***L.*** sp. Hereafter, this pure ***L.*** sp. sample, which comprises 30 individuals, will be referred to as "Mokpo".

While the Taiwan sample showed significance by the chi-square tests at the ***GPI-1****, ***MEP**** and ***PGM**** loci. Because this sample has been reported to be genetically imbalanced (Yokogawa and Tajima, 1996), this will be discussed later.

The allelic frequencies at the 28 loci of the samples are shown in Table 21. The genetic status of ***L. japonicus*** and ***L.*** sp. reported in section 3 was confirmed by the 9 newly-detected loci in this section, which exhibited replacement of major alleles between the two species at the ***FBALD-1**** and ***FBALD-2**** loci and common major alleles at the other loci (Table 21).

Regarding Table 21, samples from the Japanese archipelago except Ariake and Yatsushiro, and Hadong and Yeosu in the Korean Peninsula showed the typical genetic features of ***L. japonicus*** at the ***ADH****, ***GPI-1****, ***LDH-2**** and ***PROT-1**** loci (Table 21), at which significant differences in allelic frequencies have been detected between ***L. japonicus*** and ***L.*** sp. (Table 5).

Table 21. Allelic frequencies of sea bass samples

Locus	Allele	Tokyo	Mika-wa	Hari-ma	Suo	Yatsu-shiro	Ariake	Omura	Tottori	Mai-zuru	Ishi-kawa	Ha-dong	Yeosu	Mokpo	Yentai	Taiwan	Weihai	Zhou-shan	Fujian	Beihai
*AAT-1**	***120***	0.000	0.000	0.014	0.000	0.000	0.000	0.000	0.013	0.014	0.006	0.000	0.000	0.000	0.025	0.000				
	100	0.844	0.849	0.892	0.938	0.857	0.882	0.974	0.859	0.900	0.846	0.967	0.958	0.974	0.963	1.000				
	85	0.125	0.151	0.095	0.063	0.143	0.105	0.026	0.128	0.086	0.142	0.033	0.042	0.026	0.013	0.000				
	65	0.031	0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.000	0.006	0.000	0.000	0.000	0.000	0.000				
*AAT-2**	***-50***	0.000	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.033	0.000	0.000	0.000	0.000				
	-100	1.000	0.988	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.994	0.967	1.000	1.000	0.988	1.000				
	-150	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.000				
*ADH**	***-35***	0.010	0.000	0.014	0.000	0.000	0.000	0.000	0.000	0.029	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	-50	0.010	0.023	0.027	0.016	0.000	0.000	0.024	0.026	0.014	0.037	0.017	0.038	0.000	0.000	0.059	0.000	0.000	0.000	0.000
	-70	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.000	0.000	0.074	0.000	0.000
	-100	0.698	0.640	0.689	0.641	0.814	0.850	0.810	0.718	0.643	0.677	0.667	0.625	1.000	0.975	0.941	1.000	0.926	1.000	1.000
	-150	0.271	0.337	0.270	0.344	0.186	0.150	0.167	0.256	0.314	0.268	0.317	0.338	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	-190	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
*AK**	***120***	0.000	0.012	0.000	0.000	0.000	0.000	0.063	0.000	0.000	0.012	0.000	0.000	0.000	0.000		0.000	0.000	0.000	0.000
	100	1.000	0.988	1.000	1.000	1.000	1.000	0.906	1.000	1.000	0.988	1.000	1.000	0.978	0.988		1.000	1.000	1.000	1.000
	75	0.000	0.000	0.000	0.000	0.000	0.000	0.031	0.000	0.000	0.000	0.000	0.000	0.022	0.013		0.000	0.000	0.000	0.000
*CK**	***100***	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000				
*EST-1**	***110***	0.000	0.000	0.000	0.000	0.000	0.000	0.095	0.000	0.000	0.000	0.033	0.000	0.033	0.025					
	100	0.971	0.942	1.000	1.000	0.986	0.950	0.905	1.000	0.971	0.941	0.950	0.988	0.850	0.975					
	90	0.029	0.058	0.000	0.000	0.014	0.050	0.000	0.000	0.029	0.059	0.017	0.013	0.117	0.000					
*EST-2**	***-100***	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.988	1.000	1.000	1.000	1.000					
	-400	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.000	0.000	0.000	0.000					
*FBALD-1**	***-40***	0.000	0.000	0.000	0.000	0.129	0.125	0.000	0.000	0.000	0.006	0.083	0.025	0.900	0.800					
	-100	1.000	1.000	1.000	1.000	0.871	0.875	1.000	1.000	1.000	0.994	0.917	0.975	0.100	0.200					
*FBALD-2**	***-30***	0.000	0.000	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.017	0.050					
	-45	0.000	0.000	0.027	0.047	0.014	0.000	0.000	0.000	0.000	0.006	0.000	0.000	0.083	0.138					
	-60	0.031	0.047	0.203	0.063	0.143	0.188	0.048	0.038	0.057	0.049	0.033	0.050	0.650	0.563					
	-80	0.000	0.000	0.000	0.000	0.014	0.025	0.000	0.000	0.000	0.006	0.017	0.000	0.067	0.075					
	-100	0.969	0.953	0.757	0.891	0.829	0.788	0.952	0.962	0.943	0.939	0.950	0.938	0.183	0.175					
*G3PDH-1**	***100***	1.000	1.000	1.000	1.000	1.000	0.988	1.000	0.986	1.000	1.000	1.000	1.000	1.000	1.000	1.000				
	85	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.000				
*G3PDH-2**	***100***	0.927	0.965	0.959	0.922	0.914	0.982	0.952	0.949	0.929	0.982	0.933	0.938	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	-200	0.073	0.035	0.041	0.078	0.086	0.018	0.048	0.051	0.071	0.018	0.067	0.063	0.000	0.000	0.000	0.000	0.000	0.000	0.000
*GPI-1**	***140***	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.000	0.000	0.000	0.000	0.000
	130	0.000	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.074	0.088	0.212	0.050	0.000	0.017	0.038
	110	0.156	0.105	0.054	0.156	0.186	0.263	0.095	0.205	0.114	0.098	0.017	0.088	0.870	0.900	0.788	0.920	0.982	0.966	0.963
	105	0.000	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	100	0.833	0.872	0.932	0.844	0.814	0.738	0.905	0.795	0.886	0.902	0.950	0.913	0.056	0.000	0.000	0.030	0.019	0.017	0.000
	80	0.000	0.000	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	60	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Table 21. (Continued)

Locus	Allele	Tokyo	Mika-wa	Hari-ma	Suo	Yatsu-shiro	Ariake	Omura	Tottori	Mai-zuru	Ishi-kawa	Ha-dong	Yeosu	Mokpo	Yentai	Taiwan	Weihai	Zhou-shan	Fujian	Beihai
*GPI-2**	***-75**	0.000	0.000	0.027	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.043	0.000	0.000				
	***-100**	0.990	0.977	0.959	0.984	0.986	0.962	1.000	0.974	0.986	0.982	0.967	0.988	0.870	0.750	0.971				
	*** -200**	0.000	0.012	0.014	0.016	0.014	0.000	0.000	0.026	0.000	0.006	0.017	0.013	0.087	0.250	0.000				
	***-250**	0.010	0.012	0.000	0.000	0.000	0.038	0.000	0.000	0.014	0.012	0.017	0.000	0.000	0.000	0.029				
*IDDH**	***165**	0.106	0.081	0.097	0.117	0.114	0.115	0.225	0.132	0.100	0.093	0.117	0.175	0.083	0.113	0.098	0.020	0.000	0.069	0.000
	***140**	0.011	0.012	0.028	0.017	0.014	0.000	0.000	0.000	0.029	0.006	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	***100**	0.883	0.895	0.875	0.833	0.829	0.872	0.775	0.868	0.871	0.895	0.867	0.800	0.861	0.875	0.902	0.980	1.000	0.931	0.975
	***-50**	0.000	0.012	0.000	0.033	0.043	0.013	0.000	0.000	0.000	0.006	0.000	0.025	0.056	0.013	0.000	0.000	0.000	0.000	0.025
*IDHP-1**	***120**	0.000	0.012	0.000	0.000	0.000	0.026	0.024	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000				
	***100**	1.000	0.977	0.986	0.984	1.000	0.974	0.976	0.987	1.000	1.000	1.000	0.988	0.983	1.000	0.990				
	***70**	0.000	0.012	0.014	0.016	0.000	0.000	0.000	0.013	0.000	0.000	0.000	0.013	0.017	0.000	0.010				
*IDHP-2**	***160**	0.021	0.000	0.000	0.000	0.029	0.013	0.024	0.000	0.014	0.000	0.000	0.025	0.000	0.000	0.000	0.000	0.000	0.035	0.000
	***100**	0.979	1.000	0.959	1.000	0.971	0.988	0.976	1.000	0.986	0.994	1.000	0.975	0.983	1.000	1.000	1.000	1.000	0.966	1.000
	***60**	0.000	0.000	0.041	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.000	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000
*LDH-1**	***150**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.000	0.000	0.000	0.000	0.000				
	***100**	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.994	1.000	1.000	1.000	1.000	1.000				
*LDH-2**	***100**	0.063	0.081	0.014	0.078	0.200	0.225	0.095	0.026	0.086	0.043	0.017	0.075	0.983	0.988	1.000	1.000	1.000	1.000	1.000
	***-100**	0.938	0.919	0.986	0.922	0.800	0.775	0.905	0.974	0.914	0.957	0.983	0.925	0.017	0.013	0.000	0.000	0.000	0.000	0.000
*MDH-1**	***100**	1.000	1.000	1.000	1.000	1.000	0.946	1.000	1.000	0.971	1.000	0.983	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	***70**	0.000	0.000	0.000	0.000	0.000	0.054	0.000	0.000	0.029	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
*MDH-2**	***-100**	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*MEP**	***200**	0.000	0.023	0.000	0.000	0.014	0.000	0.000	0.038	0.029	0.006	0.000	0.000	0.033	0.000	0.000	0.000	0.000	0.000	0.000
	***150**	0.021	0.023	0.041	0.031	0.014	0.038	0.050	0.038	0.014	0.006	0.000	0.025	0.050	0.075	0.394	0.000	0.000	0.000	0.000
	***100**	0.957	0.942	0.959	0.969	0.957	0.950	0.900	0.897	0.929	0.982	0.983	0.963	0.900	0.900	0.606	1.000	1.000	1.000	1.000
	***50**	0.021	0.012	0.000	0.000	0.014	0.013	0.050	0.026	0.029	0.006	0.017	0.013	0.017	0.025	0.000	0.000	0.000	0.000	0.000
*MPI-1**	***125**	0.427	0.384	0.260	0.344	0.429	0.383	0.250	0.385	0.257	0.384	0.367	0.250	0.638	0.750	0.529				
	***100**	0.573	0.616	0.740	0.656	0.571	0.617	0.750	0.615	0.743	0.616	0.633	0.750	0.362	0.250	0.471				
*MPI-2**	***100**	1.000	0.988	0.986	1.000	1.000	1.000	1.000	1.000	1.000	0.994	1.000	1.000	0.976	1.000	1.000				
	***75**	0.000	0.012	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.000	0.000	0.024	0.000	0.000				
*PGDH**	***120**	0.000	0.000	0.000	0.000	0.014	0.013	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000				
	***100**	0.990	0.977	0.986	1.000	0.957	0.975	0.975	0.974	1.000	0.982	1.000	1.000	0.983	0.988	1.000				
	***55**	0.010	0.012	0.014	0.000	0.014	0.013	0.025	0.026	0.000	0.018	0.000	0.000	0.017	0.000	0.000				
	***40**	0.000	0.012	0.000	0.000	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.000				
*PGM**	***115**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.014	0.000	0.000	0.000	0.000	0.013	0.144	0.010	0.000	0.000	0.000
	***100**	0.448	0.453	0.541	0.484	0.386	0.363	0.405	0.436	0.471	0.402	0.500	0.588	0.283	0.363	0.519	0.265	0.278	0.349	0.350
	***75**	0.552	0.547	0.446	0.500	0.614	0.625	0.595	0.551	0.500	0.591	0.483	0.413	0.717	0.613	0.337	0.725	0.704	0.652	0.650
	***55**	0.000	0.000	0.014	0.016	0.000	0.013	0.000	0.013	0.014	0.006	0.017	0.000	0.000	0.013	0.000	0.000	0.019	0.000	0.000
*PROT-1**	***170**	0.031	0.000	0.000	0.000	0.229	0.213	0.000	0.000	0.029	0.024	0.033	0.075	1.000	1.000	1.000				
	***100**	0.969	1.000	1.000	1.000	0.771	0.788	1.000	1.000	0.971	0.976	0.967	0.925	0.000	0.000	0.000				
*PROT-2**	***-100**	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000				
*SOD-1**	***145**	0.031	0.000	0.014	0.000	0.000	0.000	0.000	0.013	0.014	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	***100**	0.969	1.000	0.986	1.000	1.000	1.000	1.000	0.987	0.986	0.994	1.000	1.000	1.000	1.000	1.000	0.980	1.000	1.000	1.000
	***20**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.000	0.000	0.000

Table 22. Nei's genetic distance (D value) between samples

	Tokyo	Mika-wa	Hari-ma	Suo	Yatsu-shiro	Ariake	Omura	Tottori	Mai-zuru	Ishi-kawa	Ha-dong	Yeosu	Mokpo	Yentai	Taiwan	Weihai	Zhou-shan	Fujian
Tokyo																		
Mikawa	0.0007																	
Harima	0.0038	0.0031																
Suo	0.0012	0.0010	0.0021															
Yatsushiro	0.0044	0.0057	0.0079	0.0057														
Ariake	0.0060	0.0072	0.0089	0.0071	0.0011													
Omura	0.0039	0.0040	0.0045	0.0034	0.0069	0.0075												
Tottori	0.0005	0.0010	0.0036	0.0013	0.0053	0.0064	0.0034											
Maizuru	0.0017	0.0011	0.0020	0.0008	0.0062	0.0073	0.0028	0.0017										
Ishikawa	0.0006	0.0004	0.0031	0.0016	0.0049	0.0064	0.0034	0.0011	0.0015									
Hadong	0.0021	0.0017	0.0028	0.0015	0.0066	0.0087	0.0037	0.0026	0.0016	0.0017								
Yeosu	0.0033	0.0028	0.0023	0.0014	0.0074	0.0091	0.0037	0.0035	0.0011	0.0034	0.0017							
Mokpo	0.1675	0.1733	0.1769	0.1696	0.1211	0.1134	0.1731	0.1702	0.1737	0.1720	0.1755	0.1734						
Yentai	0.1645	0.1713	0.1754	0.1666	0.1201	0.1132	0.1732	0.1671	0.1724	0.1706	0.1737	0.1714	0.0032					
Taiwan	0.1389	0.1457	0.1565	0.1425	0.1055	0.0980	0.1435	0.1414	0.1439	0.1482	0.1544	0.1427	0.0124	0.0134				
Weihai	0.1622	0.1694	0.1945	0.1674	0.1269	0.1083	0.1659	0.1620	0.1720	0.1739	0.1991	0.1877	0.0016	0.0026	0.0300			
Zhoushan	0.1661	0.1731	0.1988	0.1707	0.1318	0.1127	0.1719	0.1657	0.1757	0.1782	0.2036	0.1914	0.0031	0.0033	0.0312	0.0009		
Fujian	0.1649	0.1727	0.1963	0.1692	0.1299	0.1112	0.1686	0.1644	0.1745	0.1780	0.2017	0.1877	0.0019	0.0015	0.0267	0.0010	0.0014	
Beihai	0.1657	0.1730	0.1970	0.1700	0.1310	0.1122	0.1708	0.1652	0.1752	0.1785	0.2025	0.1893	0.0023	0.0019	0.0262	0.0008	0.0010	0.0004

While the Ariake sample showed rather peculiar allelic compositions at those loci as reported in section 5, also the sample from Yatsushiro which is geographically quite close to Ariake showed very similar allelic compositions to the Ariake sample (Table 21). However, another geographically close sample of Omura showed similar allelic compositions to the other samples from the Japanese archipelago except for the ***ADH**** locus, at which its allelic composition was similar to the Ariake and Yatsushiro samples (Table 21).

As for the samples from Korea, those from Hadong and Yeosu were regarded as ***L. japonicus*** from the allelic compositions at the marker loci mentioned above. But the Mokpo example comprised both ***L. japonicus*** and ***L***. sp., unlike the other Korean examples examined, most of the individuals being ***L***. sp. (Table 20).

The samples from the Chinese coast, including Taiwan, generally showed the typical genetic features of ***L***. sp. at the marker loci. Notably, the Taiwan sample showed a difference in allelic frequencies from the other Chinese samples at the ***GPI-1****, ***MEP**** and ***PGM****

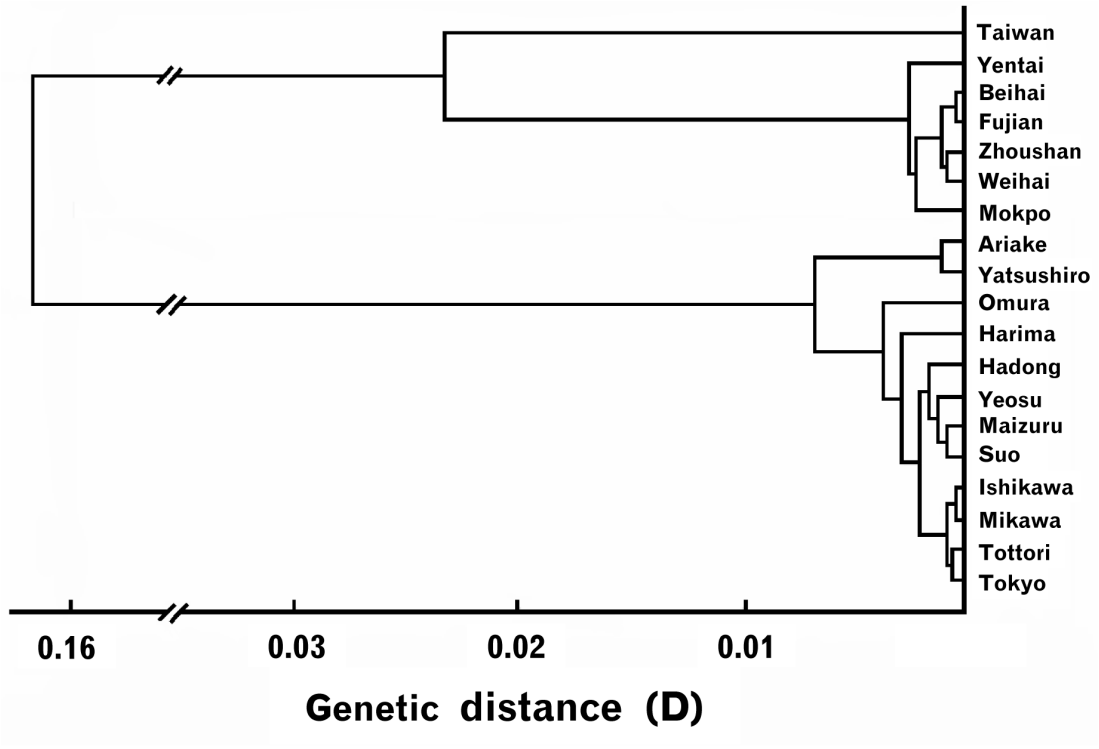

Fig. 33. Dendrogram showing genetic relationships by isozyme between samples of sea bass.

loci (Table 21).

The genetic distances (D values) between samples are shown in Table 22. A dendrogram of genetic relationships between samples based on the genetic distances (Table 22) is shown in Fig. 33. Regarding the dendrogram, there were two major clusters which could represent ***L. japonicus*** and ***L.*** sp. at the genetic distance of about 0.163, which was similar to the D value of 0.174 between the Japanese and Chinese sea bass examined in section 3.

In the ***L. japonicus*** cluster, the Ariake and Yatsushiro samples formed a distinct cluster from the others, and the Omura sample was somewhat distant from the others. While the other samples did not show any peculiar clusters according to geographical closeness, Tottori and Tokyo, plus Ishikawa and Mikawa samples for example, formed single clusters, respectively (Fig. 33). In the ***L.*** sp. cluster, the Taiwan sample was very far from the others at the distance of about 0.024. Regarding the remainders, the Beihai and Fujian, plus Zhoushan and Weihai samples formed single clusters, respectively (Fig. 33) these two clusters being connected, and in addition this united cluster was linked with the Mokpo sample, showing a correspondence in geographical proximity (Fig. 33).

Results of mitochondrial DNA

Evidence from the control region indicates the existence of two clearly distinguishable clades that correspond with ***Lateolabrax japonicus*** and ***L.*** sp. The di-

vergence between the two clades was 0.254 for the control region.

Of the 254 individuals examined, 151 belonged to ***L. japonicus*** and 103 belonged to ***L***. sp. All individuals in samples from the Japanese archipelago except Ariake and Yatsushiro, and Hadong and Yeosu in the Korean Peninsula belonged to the ***L. japonicus*** haplotypes (hereafter called J-type), and the remainders except Mokpo wholly belonged to the ***L***. sp. haplotypes (hereafter called C-type) while the Ariake, Yatsushiro and Mokpo samples comprise haplotypes belonging to both species, that is, 14 J- types and 2 C-types in Ariake, 25 J-types and 1 C-type in Ya-

Table 23. Mitochondrial DNA nucleotide substitution haplotypes of ***L .japonicus*** (J-type) by localites

Haplo-type	Ishi-kawa	Mai-zuru	Tot-tori	Omu-ra	Ari-ake	Yatsu-shiro	Suo	Kaga-wa	Mika-wa	Tokyo	Ha-dong	Yeosu	Mok-po	Total
J1	1													1
J2	1							1						2
J3	1													1
J4	1						2	1	1	1				6
J5	1		1											2
J6	1												1	2
J7	1													1
J8	1													1
J9	1													1
J10	1													1
J11		1												1
J12		1												1
J13		1	1					1						3
J14		1												1
J15		1												1
J16		1			1									2
J17		1												1
J18		1												1
J19		1		1		1				2	1	2		8
J20		1												1
J21			1											1
J22			1											1
J23			1											1
J24			1											1
J25			1											1
J26			2	1								1	1	5
J27			1						1					2
J28				1										1
J29				1										1
J30				1	1	3								5
J31				1								1		2
J32				1										1
J33				1							1			2
J34				1										1
J35				1										1
J36					1									1
J37					1									1
J38					1				1					2
J39					1	5								6
J40					1									1
J41					1									1
J42					1									1
J43					2									2
J44					1									1
J45					2									2
J46						1								1
J47						1								1
J48						1								1
J49						1								1
J50						1								1
J51						1								1
J52						1								1
J53						1								1
J54						1								1
J55						1								1
J56						1					1			2

Table 23. Continued

Haplo-type	Ishi-kawa	Mai-zuru	Tot-tori	Omu-ra	Ari-ake	Yatsu-shiro	Suo	Kaga-wa	Mika-wa	Tokyo	Ha-dong	Yeosu	Mok-po	Total
J57						1								1
J58						1								1
J59						1								1
J60						1								1
J61						1								1
J62							1							1
J63							1							1
J64							1							1
J65							1							1
J66							1							1
J67							1							1
J68							1							1
J69							1							1
J70								1						1
J71								1						1
J72								1						1
J73								1						1
J74								1						1
J75								1						1
J76								1						1
J77									1					1
J78									1					1
J79									1					1
J80									1					1
J81									1					1
J82									1					1
J83									1					1
J84										1				1
J85										1				1
J86										1				1
J87										1				1
J88										1				1
J89										1				1
J90										1				1
J91											1			1
J92											1			1
J93											1			1
J94											1			1
J95											1			1
J96											1			1
J97											1			1
J98											1			1
J99											1			1
J100											1			1
J101											1			1
J102											1			1
J103											1			1
J104											1			1
J105											1			1
J106											1			1
J107											1			1
J108											1			1
J109												1		1
J110												1		1
J111												1		1
J112												1		1
J113												1		1

tsushiro, plus 2 J-types and 8 C-types in the Mokpo samples, respectively.

The analyzed section of the control region comprised 454-455 and 457 nucleotides in ***L. japonicus*** and ***L.*** sp.**,** respectively. In ***L. japonicus***, there were 107 polymorphic sites defined by 119 substitutions: 100 transitions and 19 transversions, and 4 indels were found. One hundred and thirteen haplotypes were identified in the 151 individuals belonging to the J-type, 14 of which were shared among the samples (Table 23) while in ***L.*** sp., there were 60 polymorphic sites within the frag-

Table 24. Mitochondrial DNA nucleotide substitution haplotypes of ***L*** .sp. (C-type) by localites

Haplo-type	Beihai	Xia-men	Fu-zhou	Zhou-shan	Wei-hai	Mok-po	Ariake	Yatsu-shiro	Total
C1	1	2							3
C2	1								1
C3	1								1
C4	1								1
C5	2		1						3
C6	1								1
C7	1								1
C8	2				1				3
C9	1	1			1				3
C10	1								1
C11	1			1	2				4
C12	2								2
C13	3								3
C14	2								2
C15	3								3
C16	1			1	2				4
C17	1								1
C18		1		1					2
C19		1							1
C20		1							1
C21		1							1
C22		1							1
C23		2	1	1					4
C24		2		1					3
C25		2		1	1				4
C26		1		1					2
C27		1							1
C28			1						1
C29			1						1
C30			1						1
C31			1						1
C32			1						1
C33			1						1
C34			1						1
C35			1		1				2
C36				1					1
C37				1					1
C38				1					1
C39				1					1
C40				1					1
C41				1					1
C42				1					1
C43				1					1
C44				1					1
C45				1					1
C46				1					1
C47				1					1
C48					1	1			2
C49					1				1
C50					1				1
C51					1				1
C52					1				1
C53					1				1
C54					1				1
C55					1				1
C56					2				2
C57					1				1
C58					1				1
C59					1				1
C60					1	1			2
C61						1			1
C62						1			1
C63						2			2
C64						1			1
C65						1			1
C66							1		1
C67							1		1
C68								1	1

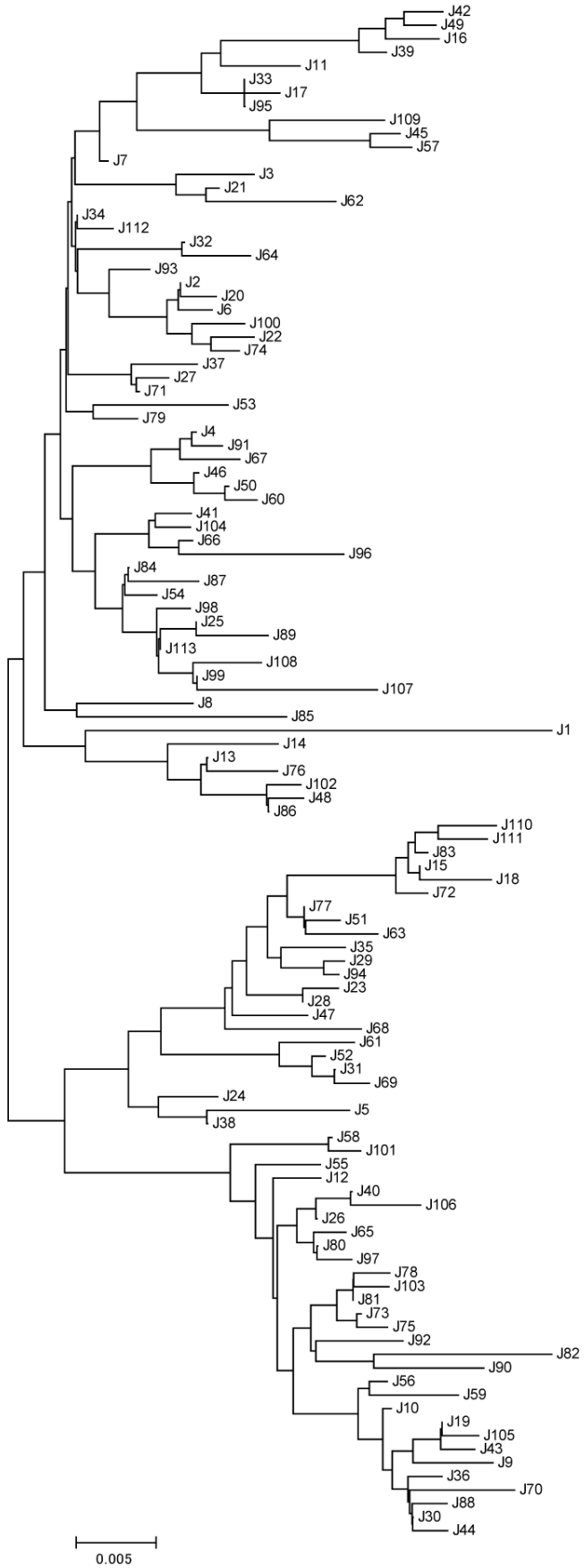

Fig. 34. Unrooted neighbor-joining tree for mitochondrial DNA control region sequences data of ***Lateolabrax japonicus*** haplotypes (J-type) based on K-2P model of substitution. Haplotypes are referred to in Table 23.

ment that we analyzed which defined 63 substitutions: 56 transitions and 7 transversions; no indels were found. Sixty-eight haplotypes were identified in the 103 individuals belonging to the C-type, 12 of which were shared among the samples (Table 24).

Among all the haplotypes, π =0.0133 and dx=6.0956 in ***L***. sp., π =0.0304 and dx=13.8677 in ***L***. ***japonicus***. The number of pairwise differences between haplotypes ranged from 1 to 20 in ***L***. sp. and 1 to 27 in ***L***. ***japonicus***. Among all the individuals, H=0.9891, π =0.0117, and dx=5.3543 in ***L***. sp. and H=0.9921, π =0.0301, and dx=13.7683 in ***L***. ***japonicus***. The transition: transversion ratio was 8.0 and 5.6 in ***L***. ***japonicus*** and ***L***. sp., respectively.

The topology of the unrooted neighbor-joining tree (Figs. 34, 35) revealed that several haplotypic clades which were moderately supported by bootstrap analysis found in the NJ tree of ***L***. ***japonicus*** (Fig. 34), however these clades did not correspond to regional populations. In ***L***. sp., no significant genealogical branches or significant clustering that correspond to sampling localities can be

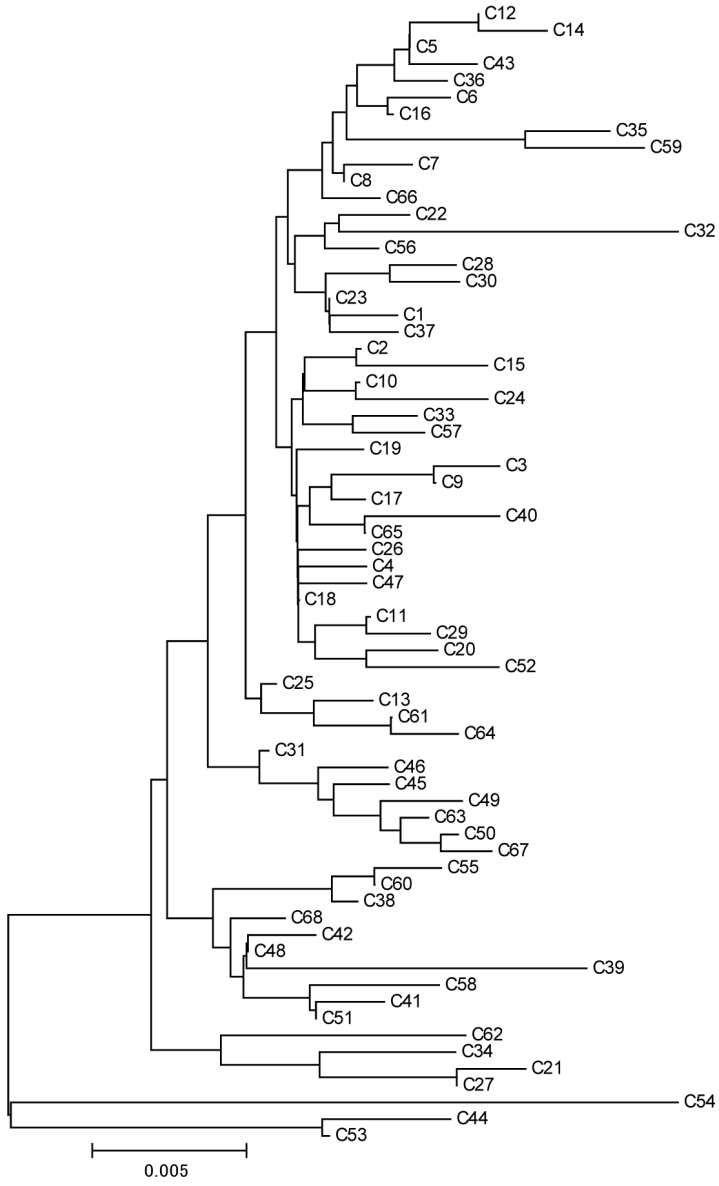

Fig. 35. Unrooted neighbor-joining tree for mitochondrial DNA control region sequences data of ***Lateolabrax*** sp. haplotypes (C-type) based on K-2P model of substitution. Haplotypes are referred to Table 24.

observed. The observed haplotypes were scattered throughout the tree (Fig. 35).

Such dendrograms calculated for regional samples of ***L. japonicus*** and ***L.*** sp. are illustrated in Figs. 36 and 37, respectively. In ***L. japonicus***, the Ariake and Yatsushiro samples formed a distinct cluster from the others, while the other examples did not show any particular clusters according to geographical proximity (Fig. 36). But in ***L.*** sp., Weihai and Mokpo, plus Beihai and Xiamen samples formed single clusters, respectively, somewhat showing a geographical cline (Fig. 37).

All populations of the two taxa showed high haplotype diversity and the minimal haplotype diversity was 0.964 (Mokpo). Nucleotide diversities varied in different populations of ***L.*** sp., ranging from 0.008 (Beihai) to 0.015 (Weihai). In general, the northern populations of ***L.*** sp. showed higher nucleotide diversity than those of southern populations. Nucleotide diversities in ***L. japonicus*** samples were higher than those of ***L.*** sp., ranging from 0.026 (Omura) to 0.035 (Maizuru) (Table 25).

The genetic structures of the populations of the two species were investigated by AMOVA (Excoffer et al., 1992). In ***L. japonicus***, when samples from Ishikawa, Maizuru and Tottori were grouped together as the Japan Sea group, samples from Omura, Ariake and Yatsushiro were referred to as the Kyushu group, samples from Suo, Harima, Mikawa and Tokyo were referred to as the Pacific group, and samples from Hadong and Yeosu were grouped together as the Korea group, most of the variation was observed to occur within populations (99.2%; P=0.296). Varia-

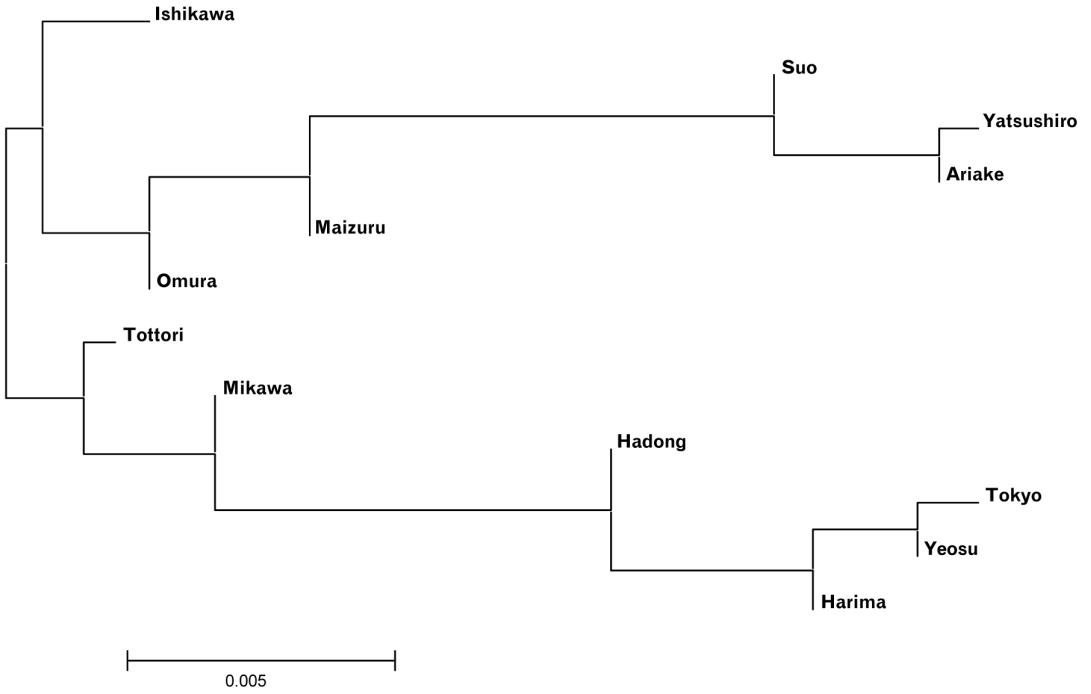

Fig. 36. Unrooted neighbor-joining tree for mitochondrial DNA control region sequences data of ***Lateolabrax japonicus*** haplotypes (J-type) by regional samples. For Ariake and Yatsushiro samples, individuals of C-type were removed for calculation.

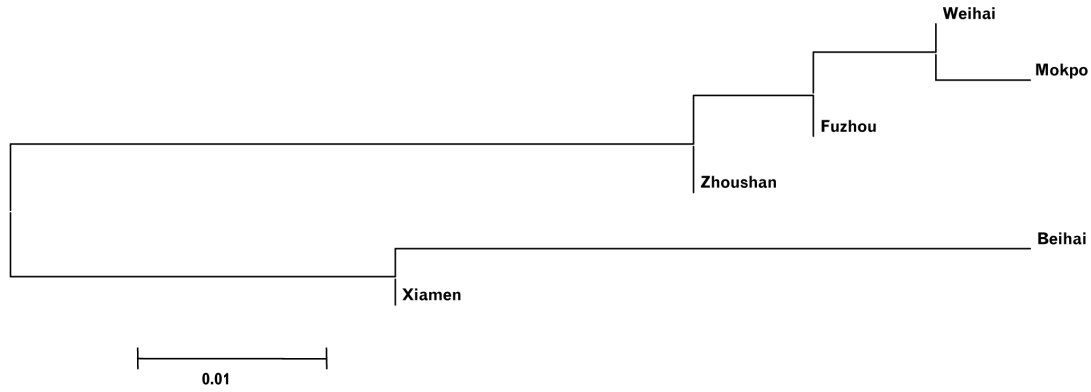

Fig. 37. Unrooted neighbor-joining tree for mitochondrial DNA control region sequences data of ***Lateolabrax*** sp. haplotypes (C-type) by regional samples. For Mokpo sample, individuals of J-type were removed for calculation.

tion among populations within groups was 0.5% (P=0.550), whereas variance among groups was 1.3% (P=0.052).

In *L*. sp., considering haplotypes, 93.7% of the genetic variation was found within populations, whereas 6.3% of the variation (P<0.001, 10000 iterations) was among samples when all 6 samples were grouped together. When the populations from Beihai, Xiamen, and Fuzhou were grouped together as a southern geographic group and the populations of Zhoushan, Weihai, and Mokpo were collected as a northern group, most of the variation (92.0%, P=0) is found within populations and a small amount (4.7%, P=0.102) separates regions. The variation among populations within groups was small (3.3%, P=0.011).

Pairwise gene flow (Nemf) values were from moderate to high in ***L. japonicus***, ranging from 7.56 (Suo vs. Ariake) to infinite in the other 25 comparisons. Among pairwise comparisons, gene flow estimates were not always the highest between nearest neighbors in ***L. japonicus***. Pairwise gene flow (Nemf) values ranged from 1.53 (Beihai vs. Mokpo) to infinite (Fuzhou vs. Zhoushan, Zhoushan vs. Weihai) for ***L.*** sp. In general, estimates of pairwise gene flow decrease when geographic distance (K) increases between ***L.*** sp. samples. Most pairwise estimates of gene flow in the two species were substantially higher than 1.0.

In ***L. japonicus***, Reduced Major Axis (RMA) regression of the log of Nemf vs. the log of the geographical distance (K) with a linear model revealed no evidence of isolation by distance. The RMA slope of the log of Nemf vs. the log of geographical distance (K) was strongly negative (b=-2.204), the matrix correlation explained very little of the variation (r=-0.124) and was not statistically significant (*Z*=407.4, P=0.183). However, ***L.*** sp. exhibited a pattern consistent with isolation by distance. The slope was strongly negative (b=-2.502) and the matrix correlation encompassed more of the variation (r=-0.656). This result was significant (*Z*=53.1, P=0.049), suggesting that ***L.*** sp. has achieved genetic equilibrium.

The mismatch distributions are unimodal for the two species respectively, characteristic of populations that have undergone large-scale expansion (Rogers and Harpending, 1992; Rogers, 1995). Mismatch distributions for all populations of the two species did not differ significantly (P>0.05) from the sudden expansion model and therefore were suitable for analysis of demographic patterns. The Fs test (Fu,

Table 25. Genetic diversity and population demographic parameters in the samples

Sample name	No.	Haplotype diversity (h)	Nucleotide diversity (π)	Fs[1]	P [2]	dx	Tau	Expansion time (YBP)
Tokyo	10	0.9778±0.0540	0.0308±0.0171	-0.9319	0.2460	14.144±6.937	18.594	1,135,200
Mikawa	10	1.0000±0.0447	0.0260±0.0145	-3.0946	0.0350	11.800±5.843	12.575	767,700
Kagawa	10	1.0000±0.0447	0.0293±0.0163	-2.7484	0.0640	13.556±6.664	16.314	996,000
Suo	10	0.9778±0.0540	0.0279±0.0155	-1.1179	0.2380	12.877±6.346	14.915	910,600
Yatsushiro[3]	25	0.9567±0.0298	0.0317±0.0164	-2.9460	0.1260	14.852±6.876	17.088	1,043,200
Ariake	14	0.9780±0.0345	0.0332±0.0177	-1.3805	0.2290	15.756±7.487	14.967	913,700
Omura	10	1.0000±0.0447	0.0256±0.0143	-3.0998	0.0540	11.782±5.834	13.867	846,600
Tottori	10	0.9778±0.0540	0.0273±0.0152	-1.2013	0.2130	12.370±6.109	14.096	860,600
Maizuru	10	1.0000±0.0447	0.0351±0.0193	-2.3744	0.0740	15.936±7.777	17.143	1,046,600
Ishikawa	10	1.0000±0.0447	0.0294±0.0163	-2.6985	0.0630	13.837±6.795	16.949	1,034,700
Hadong	21	1.0000±0.0147	0.0291±0.0152	-11.2424	0.0010	13.368±6.263	16.352	998,300
Yeosu	9	0.9722±0.0640	0.0328±0.0184	-0.2733	0.3670	15.263±7.543	17.172	1,048,400
Mokpo	8	0.9643±0.0772	0.0117±0.0072	-1.7478	0.1370	5.433±2.926	6.355	386,300
Weihai	22	0.9870±0.0175	0.0142±0.0078	-10.5277	0.0000	6.640±3.259	4.674	284,100
Zhoushan	19	1.0000±0.0171	0.0122±0.0068	-16.6301	0.0000	5.703±2.859	3.932	239,000
Fuzhou	10	1.0000±0.0447	0.0124±0.0073	-5.3790	0.0030	5.795±3.028	3.567	216,800
Xiamen	16	0.9667±0.0306	0.0093±0.0054	-4.9898	0.0050	4.313±2.253	2.276	138,300
Beihai	25	0.9667±0.0195	0.0078±0.0046	-10.0016	0.0000	3.632±1.904	3.937	239,300

[1] Fu's Fs test [2] P value of Fu's Fs statistic

[3] Individuals of C-type were ridded [4] Individuals of J-type were ridded

1997) corresponded well with the mismatch analysis. The tau value (τ), which reflects the location of the mismatch distribution crest, provides a rough estimate of the time when rapid population expansion started (Rogers and Harpending, 1992; Rogers 1995).

The tau values of the ***L. japonicus*** samples were much larger than those of ***L.*** sp., ranging from 14.50 to 16.39. The estimated expansion time for the ***L. japonicus*** samples was approximately 0.88-1.00 million years before present (Ma) assuming a divergence rate of 3.6%/Ma (Donaldson and Wilson, 1999). The tau values of ***L.*** sp. samples ranged from 2.27 to 6.36, corresponding to estimated expansion times of approximately 0.14-0.39 Ma. The tau values of northern samples were higher than those of the southern samples (Table 25).

Discussion

The genetic analysis by isozyme (Table 20) with morphological examination of the Mokpo sample revealed that it was a population mixture of ***Lateolabrax japonicus*** and ***L.*** sp., unlike the Ariake sample which is a hybridized population (see section 5). This fact could not be revealed by the mt-DNA analysis because only maternal genetic information is given from the mt-DNA gene. From the mt-DNA information only, the Ariake, Yatsushiro and Mokpo samples, which comprise the haplotypes of both species, cannot be identified as to whether they are a hybridized population or population mixture. At this point, isozymes which reflect nuclear genomic information are useful.

The fact that the Mokpo example includes both ***L. japonicus*** and ***L.*** sp. suggested that Mokpo is located in the border area of the distribution of the two species. Yokogawa (1995b) summarized the distribution of the three ***Lateolabrax*** species and showed the border of ***L. japonicus*** and ***L.*** sp. to be the southwestern edge of the Korean Peninsula. Mokpo is located in that very area (Fig. 32), corresponding well with the information. Although the two species can occur synchronously around that area, hybridization could not be recognized with the information given. This may be caused by a reproductive isolation owing to the difference in spawning season of the two species (Kim and Jun, 1997). However, hybrids between the two species can easily be produced artificially, which was successful with reciprocal combinations by male and females of the two species (Lee et al., 2000).

The allelic compositions of the Yatsushiro sample are similar to those of the Ariake sample (Table 21) which have been revealed to be a specialized population (see section 5). Further, the Yatsushiro sample included an ***L.*** sp. haplotype, the same as the Ariake one, and the chi-square tests for fitness of the Hardy-Weinberg equilibrium showed no significance at any loci. Those facts suggest that the

Yatsushiro sample is not a population mixture like the Mokpo one, being a hybridized population like the Ariake one because the Yatsushiro Sea is a geographically isolated sea like the Ariake Sea, and it is connected with the Ariake Sea by a narrow channel. Therefore, it is quite reasonable that the hybridized population of the two species like the Ariake population occurs there.

Regarding the Omura sample which is also geographically close to the Ariake Sea and in a closed sea area, its allelic composition was similar to that of the common ***L. japonicus*** samples ,unlike the Ariake and Yatsushiro ones (Table 21). In particular, in the ***PROT-1**** locus, it did not have any ****170*** alleles, which are peculiar to ***L.*** sp. (Table 21), so the sample might have originated from the hybridized population. However, the allelic composition in the ***ADH**** locus of the sample differed from the common ***L. japonicus*** samples (Table 21). The reason for this will be discussed later.

Although the hybridized populations occur in the Ariake and Yatsushiro Seas, they do not occur in the neighboring area of Omura Bay. The reason may be explained by the topography of the sea bottom. Omura Bay generally has a shallow and flat sea bottom, the depth being about 20m at most (Kamada, 1985), and it is presumed that it had emptied out during glacial periods which occasionally occurred. If the hybridized population had existed there before the glacial period, it could not have survived. Therefore, the present population could thereafter newly enter and settle there. Further, the population has been genetically isolated because of geographical isolation, which might make the allelic composition somewhat peculiar. On the other hand, if Omura Bay had been a completely closed freshwater lake, the hybridized population could not have entered.

While the Ariake and Yatsushiro Seas have some deeper zones where the depth is more than 50m (Iizuka, 1985), it did not empty out completely, even in the glacial periods, enabling the hybridized population to survive. Even though those sea areas are closed, they open into the outer sea through narrow channels, which may have enabled the populations there to have genetic interchange with the outer populations. However, mysteriously, the genetic information accumulated up to now suggests that the Ariake and Yatsushiro populations had no genetic interchange with the outer populations and that they completed their life cycle within the sea areas. The mechanism which preserves such conservative populations is very interesting, necessitating further studies.

The other examples from the Japanese archipelago and Hadong and Yeosu in the Korean Peninsula showed no particular geographic cline (Fig. 33). This tendency was also shown in the mt-DNA dendrogram (Fig. 36). Therefore, it can be regarded that the ***L. japonicus*** samples, except those from the western Kyushu

coast, are genetically almost uniform

While in the ***L***. sp. samples, it is noteworthy that the Taiwan one was very far from the remainders (Fig. 33). This was caused by differences in allelic composition of the others at the ***GPI-1****, ***MEP**** and ***PGM**** loci (Table 21). In fact, the chi-square tests for fitness of the Hardy-Weinberg equilibrium showed significance at those loci, the samples being revealed to be genetically imbalanced (Yokogawa and Tajima, 1996). The reason was that the sample was from artificial seeds (Table 19) and a genetic drift (bottle neck effect) caused the genetic imbalance. That the individuals of the sample were also peculiar morphologically may have been caused by the genetic peculiarity (Yokogawa and Tajima, 1996). Consequently, it cannot be compared with the other natural samples but the Taiwan example must be identified as ***L***. sp.

According to the isozyme and mt-DNA dendrograms, the ***L***. sp. samples showed a correspondence with the geographical cline (Figs. 33, 37), unlike the ***L***. ***japonicus*** samples, except for those from the western Kyushu coast. Also, the genetic structures of the northern and southern samples in ***L***. sp. were revealed to be different by the mt-DNA analysis. Xu et al. (2001)examined 31 isozymic loci of the northern and southern samples of sea bass, which were from Shantou, Guangdong Prov. and from Qingdao, Shandong Prov. and they calculated the D value between the two samples to be 0.008, which was somewhat larger than inter-sample level of ***L***. sp. examined in this section (Table 22).

This geographical cline may be related to the distributive range size of the two species, that is, ***L***. sp., which has greater distribution, can have such a genetic cline according to the geography. On the other hand, the difference in the genetic structure of the two species might be related. The genetic structures of the two species will be discussed later in detail, with the results of the mt-DNA analysis.

As for the results of the mt-DNA analysis of the control region, the two species apparently formed distinct clades (Figs. 34, 35). Gao et al. (2001) examined the cytoclome b region in the mt-DNA for the Japanese and Chinese sea bass by the base sequences, reporting considerable differences between the two. Those genetic differences must be caused by the specific difference of the two.

Haplotype diversities were high in both species, which is common in marine fishes (Sang et al., 1994; Jones and Quattro, 1999; Seyoum et al., 2000; Riginos and Nachman, 2001; Ishikawa et al., 2001). Nucleotide diversity in ***L***. sp. was lower than that in ***L***. ***japonicus*** despite their extensive distribution, which is consistent with isozyme diversities of previous studies (Park et al., 1996; Lou et al., 2003). The nucleotide diversity in the control region of ***L***. sp. was considerably lower than that of other marine fishes (Seyoum et al., 2000; Riginos and Nachman, 2001), while the

nucleotide diversity of ***L. japonicus*** was high.

In a qualitative assessment of demographics, ***L.*** sp. has high H and low л, a signature of rapid demographic expansion from a small effective population size, and ***L. japonicus*** has large H and large л, a characteristic of secondary contact between differentiated lineages or a large stable population with long evolutionary history (Avise, 2000). The first characteristic might apply to ***L. japonicus*** since several significant clades were found. It is suggested that the phylads evolved in isolation from each other and that present coexistence is the result of a secondary contact.

The results of mismatch distribution analysis indicated an extensive population expansion in the late Pleistocene (0.14-0.39 Ma) in ***L.*** sp., consistent with the results of the qualitative assessment of demographics based on combinations of haplotype diversity (H) and nucleotide diversity (л). Compared with ***L.*** sp., the estimates of expansion time for the populations of ***L. japonicus*** were much older (0.88-1.00 Ma), because haplotypes belonging to different significant clades were distributed in the same populations, so population expansion time might be overestimated.

Expansion times estimated for several significant lineages were 0.31－0.56 Ma, much younger than those estimated for geographical populations. The results for significant lineages also indicated a late Pleistocene expansion in ***L. japonicus***. These results suggested that the major increases in effective population size of the two temperate sea bass species are associated with a longer period of geological history, not just with the most recent glacial retreat of about 0.012 Ma (Lambeck et al., 2002).

In ***L.*** sp., the northern populations generally showed higher nucleotide diversities and earlier estimated expansion times than those of southern populations, indicating that ***L.*** sp. might have originated in the northern part of its distribution. This is consistent with the distributions of the fishes in the genus ***Lateolabrax***, the center of which was in the northern Pacific.

AMOVA analysis of ***L. japonicus*** resulted in a shallow structure, and variation among populations within regions was negative (0.46%) and not statistically significant (P=0.550) whereas the among-group variation was low (1.3%) and nearly significant (P=0.052), demonstrating that regional groups may represent genetically differentiated populations. The reason for the low and insignificant genetic differentiation among coastal populations of ***L. japonicus***, as for many other marine organisms, is most likely a fair amount of gene flow among populations (Ward et al., 1994; Wales, 1998). A statistically significant difference was found among populations within regions in the AMOVA test of ***L.*** sp. These results demonstrated that genetically differentiated populations can arise and persist in the range of the dis-

tribution of ***L***. sp.

L. ***japonicus*** had high levels of gene flow and did not exhibit a pattern of isolation by distance. The absence of isolation by distance results either when migration is so high that it overcomes the effects of genetic drift, or when there has been insufficient time following a recent expansion/contraction for a balance between migration and drift to be achieved. High levels of gene flow combined with the absence of isolation by distance tend to implicate a recent expansion (Slatkin, 1993). High levels of gene flow in the absence of isolation by distance are typically attributed to historical rather than contemporary gene flow (Patten et al., 1996; Benzine and Williams, 1997).

Gene flow in ***L***. sp. was from moderate to high, and the plot of the log of Nemf vs. the log of the geographical distance (K) revealed strong isolation by distance, indicating that ***L***. sp. is in a genetic equilibrium. If the gene flow and isolation by distance are observed, it is more likely that the observed patterns are a result of recent processes rather than historical association (Barber, 1999).

Acknowledgments

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8 Specific divergence of three *Lateolabrax* species

The study in the previous sections revealed problems between ***Lateolabrax japonicus*** and ***L***. sp., which was newly recognized as an independent species and there is one more species of ***Lateolabrax latus*** which was described by Katayama (1957) in the genus ***Lateolabrax***. At this point, three valid species of ***Lateolabrax*** are recognized. In this section, the genetic characteristics of the three species were examined using isozyme analysis and their patterns of divergence were discussed.

Materials and methods

Lateolabrax japonicus specimens used in this study comprised of 37 individuals, 21 caught in December, 1995, and 16 caught in December, 1996, both by trawling in the southern Harima Sea, Seto Inland Sea. Forty examples of ***L***. sp. were obtained in November, 1995, from cultured fish transported from Yentai, Shandong Province, China, to Hiketa nursery, Kagawa, in spring 1995. ***L. latus*** specimens comprised of 10 individuals, 1 caught in November, 1993 at off Kamaguchi, Awaji Island, Seto Inland Sea, by angling, the others caught in coastal waters off Tsubakidomari, Tokushima, by fixed shore net (3 in January-March, 1993, and the other 6 in January-March, 1995, respectively).

Isozymes were examined with the common methods noted at section 2. As the genetic markers, all of the 29 loci which were shown in Table 1 were used. Genetic distances (D values) after Nei (1990) were calculated from the allelic frequencies of the three species. Unbiased values for each D value were calculated after Nei (1990) and 95% confidence intervals according to each unbiased D value were calculated after Shinjo (1986). In addition, years of divergence according to the D values were given after Nei (1990).

Results

Allelic frequencies of the 29 loci in the three species with values to indicate their genetic characteristics are shown in Table 26, and electrophoretograms of some significant isozymes were illustrated in Fig. 38. ***Lateolabrax latus*** showed complete replacement of alleles, compared with ***L. japonicus*** and ***L***. sp., at the ***ADH****, ***CK****, ***EST-2****, ***GPI-1****, ***PGM****, ***PROT-1**** and ***SOD-1**** loci, and replacement of a major allele at the ***MEP**** locus (Fig. 38, Table 26).

Table 26. Allelic frequencies with values to indicate genetic characteristics of three species of ***Lateolabrax***

Locus	Allele	Frequency		
		L. japonicus	***L.* sp.**	***L. latus***
AAT-1*	****120***	0.014	0.025	0.000
	****100***	0.892	0.963	1.000
	****85***	0.095	0.013	0.000
AAT-2*	****-100***	1.000	0.988	1.000
	****-150***	0.000	0.013	0.000
ADH*	****-35***	0.014	0.000	0.000
	****-40***	0.000	0.000	1.000
	****-50***	0.027	0.000	0.000
	****-70***	0.000	0.025	0.000
	****-100***	0.689	0.975	0.000
	****-150***	0.270	0.000	0.000
AK*	****100***	1.000	0.988	1.000
	****75***	0.000	0.013	0.000
CK*	****100***	1.000	1.000	0.000
	****55***	0.000	0.000	1.000
EST-1*	****110***	0.000	0.025	0.000
	****100***	1.000	0.975	1.000
EST-2*	****250***	0.000	0.000	1.000
	****-100***	1.000	1.000	0.000
FBALD-1*	****-40***	0.000	0.800	0.000
	****-100***	1.000	0.200	0.950
	****-120***	0.000	0.000	0.050
FBALD-2*	****-30***	0.014	0.050	0.000
	****-45***	0.027	0.138	0.000
	****-60***	0.203	0.563	0.000
	****-80***	0.000	0.075	0.000
	****-100***	0.757	0.175	1.000
G3PDH-1*	****100***	1.000	1.000	1.000
G3PDH-2*	****100***	0.959	1.000	1.000
	****-200***	0.041	0.000	0.000
GPI-1*	****140***	0.000	0.013	0.000
	****130***	0.000	0.088	0.000
	****110***	0.054	0.900	0.000
	****100***	0.932	0.000	0.000
	****90***	0.000	0.000	1.000
	****80***	0.014	0.000	0.000
GPI-2*	****-75***	0.027	0.000	0.050
	****-100***	0.959	0.750	0.950
	****-200***	0.014	0.250	0.000
IDDH*	****165***	0.097	0.113	0.200
	****140***	0.028	0.000	0.800
	****100***	0.875	0.875	0.000
	****-50***	0.000	0.013	0.000

Locus	Allele	Frequency		
		L. japonicus	***L.* sp.**	***L. latus***
IDHP-1*	****120***	0.000	0.000	0.050
	****100***	0.986	1.000	0.950
	****70***	0.014	0.000	0.000
IDHP-2*	****100***	0.959	1.000	1.000
	****60***	0.041	0.000	0.000
LDH-1*	****100***	0.100	1.000	1.000
LDH-2*	****100***	0.014	0.988	1.000
	****-100***	0.986	0.013	0.000
MDH-1*	****100***	1.000	1.000	1.000
MDH-2*	****-100***	1.000	1.000	1.000
MEP*	****150***	0.041	0.075	0.950
	****100***	0.959	0.900	0.050
	****50***	0.000	0.025	0.000
MPI-1*	****135***	0.000	0.000	0.063
	****125***	0.260	0.750	0.000
	****100***	0.740	0.250	0.938
MPI-2*	****100***	0.986	1.000	1.000
	****75***	0.014	0.000	0.000
PGDH*	****100***	0.986	0.988	0.900
	****55***	0.014	0.000	0.100
	****40***	0.000	0.013	0.000
PGM*	****115***	0.000	0.013	0.000
	****100***	0.541	0.363	0.000
	****95***	0.000	0.000	1.000
	****75***	0.446	0.613	0.000
	****55***	0.014	0.013	0.000
PROT-1*	****170***	0.000	1.000	0.000
	****100***	1.000	0.000	0.000
PROT-2*	****-100***	1.000	1.000	1.000
SOD-1*	****145***	0.014	0.000	0.000
	****100***	0.986	1.000	0.000
	****95***	0.000	0.000	1.000
SOD-2*	****-100***	1.000	1.000	1.000
Alleles/Locus		1.893	1.857	1.286
P*		0.250	0.286	0.250
P		0.321	0.250	0.000
P+P*		0.571	0.536	0.250
Average	Ho	0.095	0.109	0.033
Hetero-	He	0.097	0.112	0.036
zygosity	Ho/He	0.978	0.975	0.928

The genetic distances (D values) between the three species and a dendrogram based on unbiased genetic distances plus a time scale (Nei, 1990) are given in Table 27 and Fig. 39, respectively. ***L. latus*** was found to be somewhat closer to ***L. japonicus*** rather than to ***L.*** sp. in genetic distance (Table 27), owing to the existence

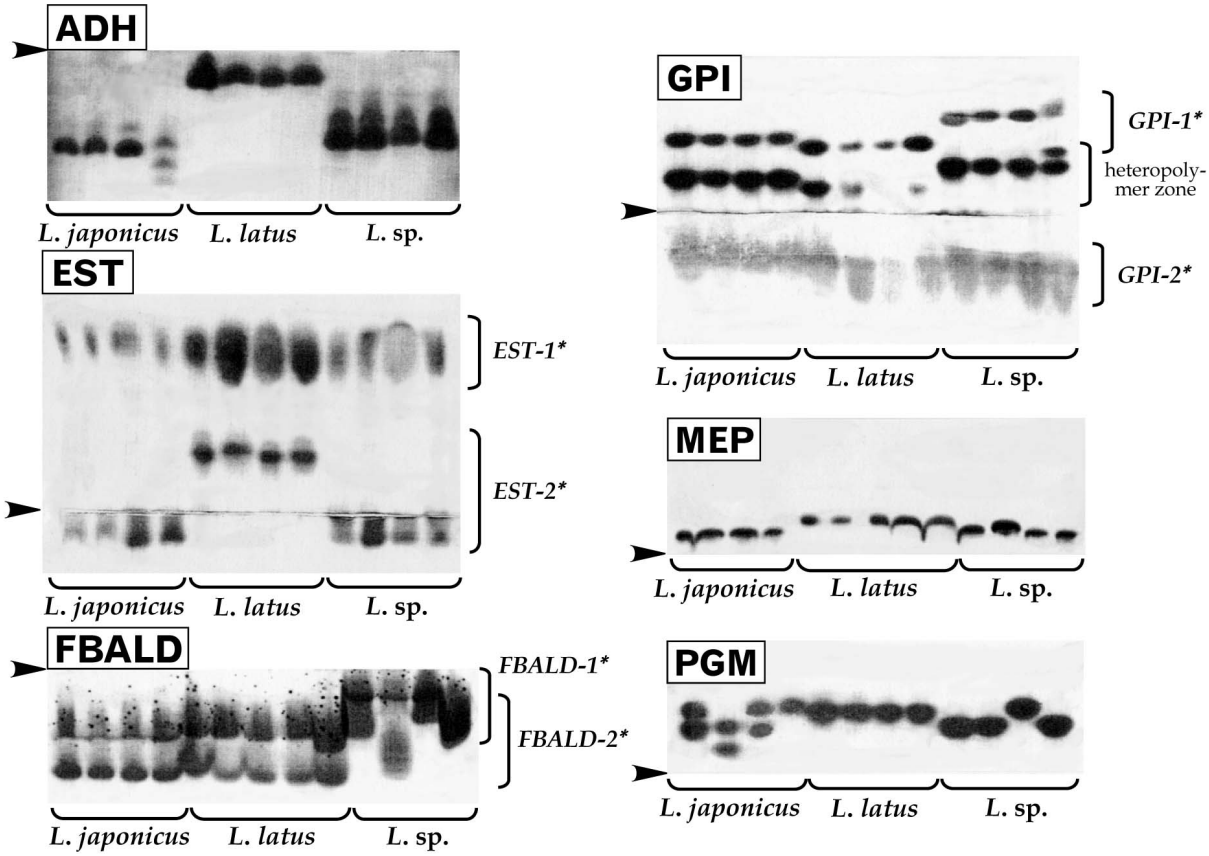

Fig. 38. Selected electrophoretograms of some isozymes in three species of ***Lateolabrax***.

of alleles common only to the two former species at two loci (***FBALD-1****** and ***FBALD-2****), compared with a single major allele common only to ***L. latus*** and *L.* sp. at just one loci (***LDH-2****) (Table 1). The dendrogram shows that ***L. japonicus*** and *L.* sp. form a single cluster, of genetic distance about 0.18 (0.9 Ma). The genetic distance of that cluster from ***L. latus*** was about 0.44 (2.2 Ma) (Fig. 39).

Discussion

Park et al. (1996) examined isozymes including loci in the general protein (PROT) of ***Lateolabrax japonicus*** and *L.* sp. from Korea and they state that *L.* sp. does not possess the ***Pt-4*** locus which is apparent in ***L. japonicus***. Regarding the electrophoretogram of PROT (they referred to it as PT) which they showed, the ***Pt-4*** locus appeared at the lowest end of the cathodal zone. In fact, in PROT, the band zones which appear at that position are not PROT but an enzyme of fructose biphosphate aldolase (FBALD) (Fig. 38). Also, their recognition of the ***Pt-2*** locus which appeared in the anodal zone, being close to the origin, can be bands of

Table 27. Interspecific genetic distances (D values) in ***Lateolabrax***

	L. japonicus	*L.* sp.
L. japonicus		
L. sp.	0.1681	
L. latus	0.3695	0.4110

creatine kinase (CK) (Figs. 12, 23). These two enzymes dye well with amide black 10B which is also used to dye PROT. It was experimentally recognized by the author by comparing the band patterns in PROT with the two enzymes. Although Park et al. (1996) thought that *L*. sp. lacked the ***Pt-4*** (presumed to be ***FBALD-2**** in this study) locus, in regard to their electrophoretogram, that locus can be recognized also in *L*. sp., including 4 heterozygotes, and the bands of the remainders are presumed to be overlapped with those of the upper zone which is ruled by a different locus. On the other hand, the FBALD bands are not obvious in smaller individuals because the activity of the enzyme is not high.

The ***Lateolabrax japonicus*** specimens examined at this section showed a similar genetic composition to those studied at section 3, the specimens in both studies having been obtained from the same area of the Harima Sea. The genetic distance between the two *L. japonicus* samples was 0.0027, a value slightly larger than the inter-population level of *L. japonicus* in the Harima Sea, according to Ohtani et al. (1997), but here regarded as being within the local population level for *L. japonicus*, except the Ariake Sea population, as summarized at section 5.

The genetic relationships among the three ***Lateolabrax*** species obtained in this study suggested a specific divergent process as follows: The common ancestral species of the three present species existed before 3 Ma. *L. latus* diverged initially, at about 2.2 Ma (middle Pliocene). Thereafter, *L. japonicus* and *L*. sp. diverged at about 0.9 Ma (early Pleistocene) (Fig. 39).

The fact that *L. latus* is distributed only in coastal waters around southern Japan (Katayama, 1960a, 1960b, 1965b) suggests that initial divergence occurred in Japanese waters. At that time (mid-Pliocene), it has been hypothesized that the Japan Sea was a semi-closed area, being strongly influenced by a cold current

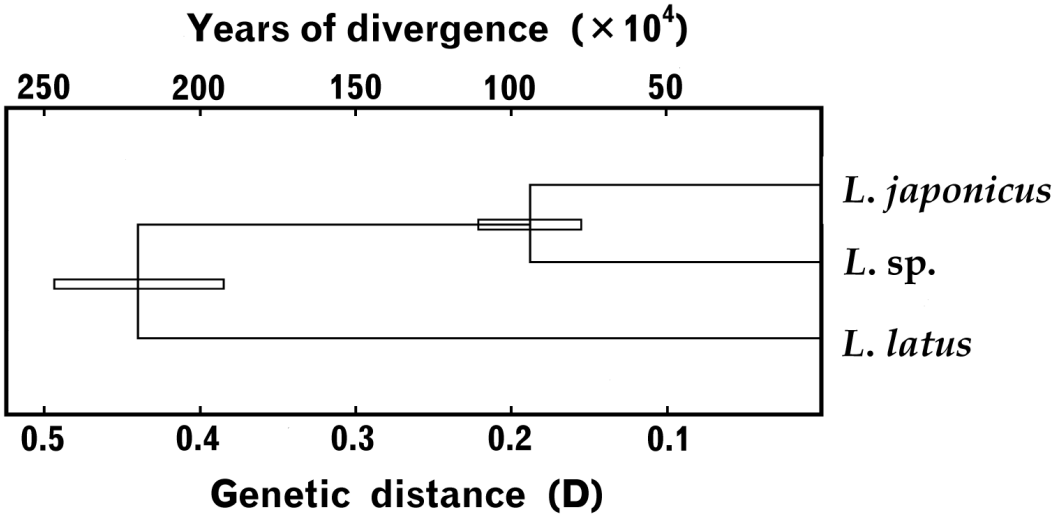

Fig. 39. Dendrogram based on unbiased genetic distances between three species of ***Lateolabrax***. Open bars indicate 95% confidence intervals.

(Kaseno, 1975, 1994), with, concurrently, a huge enclosed sea existing in the region of the present East China Sea (Kizaki and Oshiro, 1977; Kimura, 1991). The specific divergence of ***L. latus*** may have resulted from reproductive isolation in such enclosed waters.

Thereafter, in the early Pleistocene, the Korea Channel formed, subsequently closing and opening repeatedly in step with the eustacies by cyclic occurrence of glacial and inter-glacial epochs (Kaseno, 1994; Kitamura and Kondo, 1990; Nishimura, 1990; Kitamura et al., 1993; Oba, 1993). During this period, ***L. japonicus*** and ***L.*** sp. may have diverged owing to repeated isolation of populations. Nishimura（1990）hypothesized that some present marine species in the Japan Sea were established in this period following reproductive isolation caused by the closing of the Korea Channel. During repeated diastrophisms, newly-divergent ***L. japonicus*** and ***L.*** sp. may have remained genetically mixed in some waters, now being represented by a relictual hybrid population. The Ariake form of ***L. japonicus***, a specialized population (Kinoshita et al., 1995; also, see section 5), can be regarded as an example of such. Further, relictual gene flow following hybridization may be evidenced by the presence in some young ***L. japonicus*** of black dots, being a typical morphological characteristic of ***L.*** sp. (see section 6).

Yokogawa (1995) summarized some ecological characteristics of the three ***Lateolabrax*** species, showing the general tendencies in each. ***L.*** sp. has a rather strong tolerance of fresh water, often entering rivers, sometimes as far as the upper zone. ***L. latus***, on the other hand prefers coastal rocky areas bordering the open sea, and hardly enters rivers. ***L. japonicus*** appeared to be an intermediate between ***L. latus*** and ***L.*** sp.

A proposal that ***L.*** sp. is systematically closest to the common ancestral ***Lateolabrax***, on the basis of its having the widest distribution of the present three species, leads to the suggestion that an ancestral population of ***L.*** sp. become removed from a less saline habitat to the open sea, subsequently giving rise to ***L. latus***. Habitat changes can be postulated, since the fresh waters which were initially utilized diminished in area. In addition, during colder periods, fresh water avoidance, owing to the lower temperatures than in sea water, could also have been a factor. The earlier-mentioned hypothesis that ***L. latus*** was established in the Japan Sea, during colder Pliocene climates (Kaseno, 1975, 1994) may be significant. A second specific divergence, that of ***L. japonicus***, might be explained on the similar grounds.

On the other hand, if ***L. latus*** is the closest to the common ancestral species, divergence of populations, that had become isolated in fresh or estuarine waters may have resulted in subsequent speciation. At this time, however, in the absence of, for example, fossil evidence, further speculation is unwarranted.

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9 Summary

Studies on the biological characteristics and specific divergence of species in sea bass, genus ***Lateolabrax,*** were carried out.

Morphological and genetic differences between Japanese and Chinese forms of the sea bass showed pronounced differences in some morphological characters, in particular the number of pored lateral line scales, gill rakers and vertebrae, which differed sufficiently for unequivocal differentiation of the forms, when used in combination. Isozyme analyses of genetic characters indicated a complete replacement of alleles at the ***PROT-1**** locus, and extreme differences in allelic frequencies at the ***GPI-1**** and ***LDH-2**** loci. The genetic distance (D value) between the Japanese and Chinese forms, calculated from isozymic allele frequencies, was 0.174, a figure significant at the inter-specific level. The considerable morphological and genetic differences suggested that the sea bass from China is a distinct species from ***L. japonicus***.

The morphological and genetic features of the so-called "hoshisuzuki", which were reported from around western Japan and were similar to the cultured Chinese sea bass ***Lateolabrax*** sp., were examined. The morphological characteristics of "hoshisuzuki" corresponded well with the range of ***L***. sp. in the number of pored lateral line scales, gill rakers and vertebrae. The genetic characteristics of "hoshisuzuki" also corresponded well with those of ***L***. sp. at the significant loci

where a considerable difference in allelic compositions between ***L. japonicus*** and ***L.*** sp. have been detected, such as the ***ADH**, **GPI-1**, **LDH-2**** and ***PROT-1**. The results suggested that the so-called "hoshisuzuki" was the imported Chinese sea bass ***Lateolabrax*** sp., which escaped from culture cages and matured.

Sea bass from the Ariake Sea, characterized by black dots on the lateral body region as in the Chinese sea bass, ***L.*** sp., were examined and compared morphologically and genetically with ***L. japonicus*** and ***L.*** sp. Some meristic characters of the Ariake form tended to fall midway between values for the two former species. Genetic features, evaluated by isozyme analyses, indicated that the Ariake form as represented a simple Mendelian population, there being no significant differences from a Hardy-Weinberg equilibrium according to chi-square tests. Although some extreme differences in allelic frequencies were found at some loci between ***L. japonicus*** and ***L.*** sp., the Ariake form possessed many heterozygotes at the ***PROT-1**** locus, in addition to allelic frequencies at some loci conforming to those of ***L.*** sp. Average allele numbers per locus, rate of polymorphic loci and average heterozygosity of the Ariake form were higher than for either ***L. japonicus*** or ***L.*** sp., indicating high genetic variation in the former. The results suggested that the Ariake population is genetically independent of other populations of ***L. japonicus***, but might be genetically influenced by ***L.*** sp.

The dotted types of Japanese sea bass ***L. japonicus,*** including those in the Ariake form, which are somewhat similar to ***L.*** sp. were examined. Morphologically, the dotted type significantly differed from the non-dotted type in some of the characters. The average values of proportion and meristic counts of the dotted type inclined to those of ***L.*** sp. Genetically, the allelic composition of the dotted type was rather closer to ***L.*** sp. than ***L. japonicus,*** and the same for the morphometrics. The results suggest that the dots appear in individuals which have genes peculiar to ***L.*** sp. in the ***L. japonicus*** population, and they morphologically appear to be close to ***L.*** sp. The genetic influence of ***L.*** sp. is caused by the possibilities that the genes peculiar to ***L.*** sp. survive during the specific divergence process, or that genetic introgression of ***L.*** sp. to the ***L. japonicus*** population occurs after the specific divergence.

Genetic variations in local populations of ***L. japonicus*** and ***L.*** sp. around east Asian coasts including Japan, Korea, Taiwan and China were examined by use of isozymes and mitochondrial DNA (mt-DNA) as genetic markers. The genetic analysis by isozyme with morphological examination of the Mokpo example revealed that it was a population mixture of ***Lateolabrax japonicus*** and ***L.*** sp., unlike the Ariake population. This fact suggested that Mokpo, where it is located at the southwestern edge of the Korean Peninsula, is in the border area of distribution of

the two species. The allelic compositions of the Yatsushiro sample are similar to those of the Ariake sample, suggesting that it is a hybridized population between ***L. japonicus*** and ***L***. sp., the same as the Ariake population. The other samples from the Japanese archipelago, except Omura Bay, and Hadong and Yeosu in the Korean Peninsula showed no particular geographic cline. This tendency was also shown in the mt-DNA dendrogram. But in the ***L***. sp. samples, the Taiwanese sample was very far from the remainders. This was caused by a genetic imbalance owing to artificial production. According to the dendrograms of the isozyme and mt-DNA, the ***L***. sp. samples showed a geographic cline, unlike the ***L***. ***japonicus*** samples except for those from the western Kyushu coast.

The genetic analysis of the sequence data from the mt-DNA control region showed two distinct clades consistent with the two species. The demographic history of the two species using Fu's Fs neutrality tests and mismatch distribution analysis suggested an ancient population expansion for both species. A northern origin of ***L***. sp. was suggested by analyzing nucleotide diversities and population expansion times. The results of AMOVA revealed no population structure at all hierarchical levels for ***L***. ***japonicus***. A shallow but highly significant genetic differentiation was observed among populations within regions in ***L***. sp., demonstrating that genetically differentiated populations can arise and persist in the range of ***L***. sp. Moderate to high estimates of gene flow were obtained for populations of the two species. There was no detectable isolation by distance in ***L***. ***japonicus***, which is probably the result of a recent expansion. Isolation by distance was observed in ***L***. sp., suggesting that ***L***. sp. is in a genetic equilibrium.

Genetic divergence of three species of genus ***Lateolabrax*** (***L***. ***japonicus***, ***L***. sp. and ***L***. ***latus***), was examined by isozyme analysis. ***L***. ***latus*** exhibited complete replacement of alleles both with ***L***. ***japonicus*** and ***L***. sp., at the ***ADH****, ***CK****, ***EST-2****, ***GPI-1****, ***PGM****, ***PROT-1**** and ***SOD-1**** loci, plus a considerable difference in allelic frequencies at the ***MEP**** locus, indicating a greater divergence than the interspecific level appeared between ***L***. ***japonicus***, and ***L***. sp. ***L***. ***latus*** was somewhat closer to ***L***. ***japonicus*** rather than ***L***. sp. in genetic distance, because the common major alleles occurred only with ***L***. ***japonicus*** at two loci (***FBALD-1**** and ***FBALD-2****), compared with a single major allele common with ***L.*** sp. at just one loci (***LDH-2****). The genetic information suggested that ***L***. ***latus*** represented an early offshoot from the common ancestor of ***Lateolabrax***, ***L***. ***japonicus*** and ***L***. sp. diverged considerably later.

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