Genetic Differentiation between Two Types of Dark Chub, *Zacco temmincki*, in Japan

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Abstract   Two types of the dark chub, *Zacco temmincki*, collected from 10 river systems in Japan were genetically characterized at 27 protein coding loci using starch-gel electrophoresis. They were fixed for different alleles at 13 loci. No hybrid individuals were observed, even in specimens collected in stations where both types appear sympatrically, indicating that each type of the dark chub represents a distinct species.

The dark chub, *Zacco temmincki*, is widely distributed from Korea to the western parts of Japan (Uchida, 1939; Bănărescu, 1968; Nakamura, 1969). We recognized two different types, here called Type A and Type B, in Japanese populations based on some morphological differences (Watanabe and Mizuguchi, 1988). The fish of Type A possesses a transparent dorsal fin with a faint red blotch and their front edge of the pectoral fin is red, whereas the dorsal fin of Type B is dark yellow. The number of pored scales of Type A tends to be larger than that of Type B. Type B is distributed more widely in the western part of Japan than Type A, which extends from Tokai, through Lake Biwa to the Sanyo District along the Seto Inland Sea. The overall abundance of Type A is far less than that of Type B.

We have examined morphological, genetic and taxonomic aspects of these two types. This paper deals with the biochemical population genetics of 17 populations of the dark chub collected from 10 river systems in Japan. The purpose of the present study is to define the magnitude of genetic difference between the two types. Allozyme analysis suggested that the dark chub distributed in the Korean Peninsula also comprises two distinct species, the so-called MM and MS types (Yang and Min, 1987). The purpose of the present study also includes suggestion of a relationship between Japanese and Korean dark chub populations.

Materials and methods

Samples were collected from 10 river systems in middle and western areas of Honshu, Japan, by hand net and trap bottle during the period from 1988 to 1990. Sample locations are shown in Fig. 1 and sample size is listed in Table 2. Whole fish bodies were frozen immediately following collection until processed for electrophoresis. Horizontal starch-gel protocols followed May et al. (1979) and Harris and Hopkinson (1976). An initial identification between Types A and B was made based on the difference in color patterns of the dorsal and pectoral fins.

A list of enzyme stains used, their abbreviations, Enzyme Commission number, the numbers of loci expressed and others are given in Table 1. Tissues of the liver, skeletal muscle, eye ball and heart were used. Locus and gene nomenclatures follow Allendorf and Utter (1979) and Shaklee et al. (1990). If multiple loci coded for a protein, the loci were numbered starting with the one which encoded the protein with the least anodal migration. The most common allele at a locus of the B type was designated *100, and other alleles were assigned numbers according to the mobility of their homomeric protein products relative to that of the *100 allele.

Homology of the loci which showed different mobilities on the gels for each type and were considered to be fixed for different alleles were confirmed
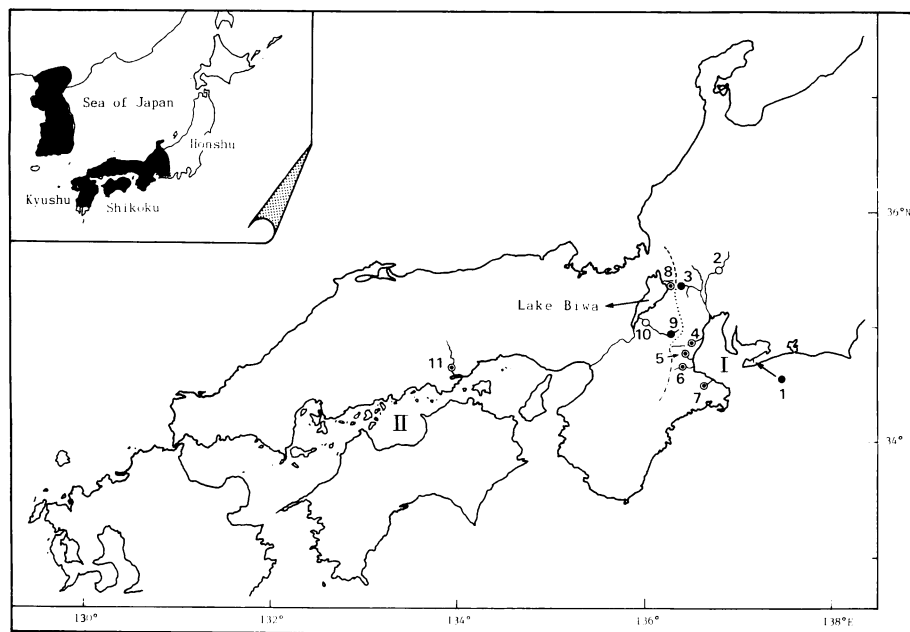
Fig. 1.　Map of Japan and the Korean Peninsula showing the sites where dark chub were sampled (○; Type A, ●; Type B, ◉; Types A and B) and its endemic range (shaded; Uchida, 1939, Bănărescu, 1968; Nakamura, 1969).
1; Shinbori River, 2; Nagara R., 3; Ibi R., 4; Suzuka R., 5; Ano R., 6; Kumozu R., 7; Tokida R., 8; Ane R., 9, 10; Yasu R., 11; Asahi R., I; Ise Bay, II; Seto Inland Sea. ----; Ibuki Mountains, ·······; Suzuka Mountains, –··–··; Nunobiki Mountains.

by breeding tests. For the remaining loci, the Mendelian nature of the electrophoretic variants was inferred from the banding patterns on the gels according to the following criteria: (1) Banding patterns had to be consistent with the known molecular structure of that protein. (2) When a gene was expressed in more than one tissue, variant phenotypes had to be parallel among tissues (Grant et al., 1983).

Results

Among 27 presumative loci surveyed, 7 loci were polymorphic in the Japanese dark chub collections following the criterion that a locus is considered polymorphic in populations in which the frequency of the most common allele is less than 0.99.

At 13 loci, Type A was fixed for different alleles from those of Type B (Fig. 2). Allelic frequencies of 17 dark chub populations collected from Japanese rivers are presented in Table 2. At all of the polymorphic loci, the deviation of observed number from

the expected number was not significant (assuming Hardy-Weinberg equilibrium). Nei's (1978) genetic distance value (D; revised by sample size) using 27 loci was 0.64 between Types A and B when the total collections were combined.

In Type B, the $PEPA*$ locus was almost fixed for different alleles for the east and west collections divided at the Ibuki, Suzuka and Nunobiki Mountains. Similarly, the $PEPC*$ locus of Type A was almost fixed for different alleles for each collections. Allelic variation at the $Ak*$ locus was observed exclusively in Type B from the Ano and Kumozu River populations.

Discussion

According to our observations, Type A tends to be distributed in the lower reaches and more placid waters compared to Type B. In some stations, however, both types are distributed sympatrically and sometimes they were caught together in a single trap bottle. The lack of hybrid specimens showing

up in the analysis clearly indicates that Types A and B are distinct species. The *D* value obtained (0.64) between both types corresponds to the range of values obtained at the interspecific level for fish species by other workers (Buth and Burr, 1978; Shaklee et al., 1982).

In the Korean Peninsula, the MS type is distributed throughout the southern parts of the peninsula, while the range of the MM type is almost entirely confined to the southeast part of the peninsula (Yang and Min, 1989). It is difficult to immediately identify relationships between the Japanese and Korean forms, due to the lack of allozyme patterns shown for the Korean congener, except for the *MDH** locus (Yang and Min, 1989). Judging from the electromorphs of the *MDH** locus, the Korean MM type is

not identical with both Japanese types. Based on some morphological characters (Yang and Min, 1987), it is considered that both Korean types are similar to Type B in Japan. This suggests that the dark chub consists of at least three distinct species, though a comparative study needs to be conducted on all of the Korean and Japanese forms.

It is suggestive that the east and west collections divided at the Ibuki, Suzuka and Nunobiki Mountains were almost fixed for different alleles in both types, respectively. This phenomenon is presumably a reflection of the isolation between the east and west populations in the mountains, which form the watersheds of the rivers entering Ise Bay and the Seto Inland Sea. In earlier times, however, it is supposed that the outlet of Lake Biwa had been Ise Bay, but

Table 1. Electrophoretic analysis of dark chub from Japan.

Enzyme	Enzyme number	Locus	Tissue[a]	Buffer[b]
Aspartate aminotransferase	2.6.1.1	*AAT-1**	E, L, M	3, 4
		AAT-2*	E, H, L, M	3, 4
Acid phosphatase	3.1.3.2	*ACP**	L	3, 4
Adenylate kinase	2.7.4.3	*AK**	M	3
Creatine kinase	2.7.3.2	*CK**	M	1
Esterase	3.1.1.-	*EST**	L	3
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	*GAPDH**	M	3
Glycerol-3-phosphate dehydrogenase	1.1.1.8	*G3PDH-1**	M	3
		G3PDH-2*	M	3
Glucose-6-phosphate isomerase	5.3.1.9	*GPI-1**	M	1, 3
		GPI-2*	M	1, 3
Glutathione reductase	1.6.4.2	*GR**	L	1
Isocitrate dehydrogenase (NADP⁺)[c]	1.1.1.42	*IDHP-1**	M	3, 4
		IDHP-2*	L	3, 4
		IDHP-3*	L	3, 4
L-Lactate dehydrogenase	1.1.1.27	*LDH-1**	L	3, 4
		LDH-2*	E, H, M	3, 4
		LDH-3*	E, H, M	3, 4
Malate dehydrogenase	1.1.1.37	*mMDH-1**	H, M, L	3
		sMDH-2*	E, H, L	3
		sMDH-3*	E, H, M	3
Malic enzyme (NADP⁺)	1.1.1.40	*MEP**	M	4
Dipeptidase	3.4.-.-	*PEPA**	E, M	1, 2
Peptidase-C	3.4.-.-	*PEPC**	E, M	2
Phosphogluconate dehydrogenase	1.1.1.44	*PGDH**	M	3
Phosphoglucomutase	5.4.2.2	*PGM**	M	4
Superoxide dismutase	1.15.1.1	*SOD**	L	2, 3, 4

[a] E=eye, H=heart, L=liver, M=muscle.

[b] 1=a discontinuous Tris-citric acid (gel pH 8.5), lithium hydroxide-boric acid (tray pH 8.5) buffer system described by Ridgway et al. (1970).

2=a Tris-boric acid-EDTA buffer (pH 8.5) described by Markert and Faulhaber (1965).

3=an amine (N-(3-Aminopropyl)-morpholine) citrate buffer (pH 6.5) described by Clayton and Tretiak (1972).

4=an amine (N-(3-Aminopropyl)-diethanolamine) citrate buffer (pH 7.0) described by Numachi et al. (1979) with slight modification based on the Clayton and Tretiac (1972).

[c] Homology of the *IDHP-2** and *-3** loci between Types A and B is tentative.
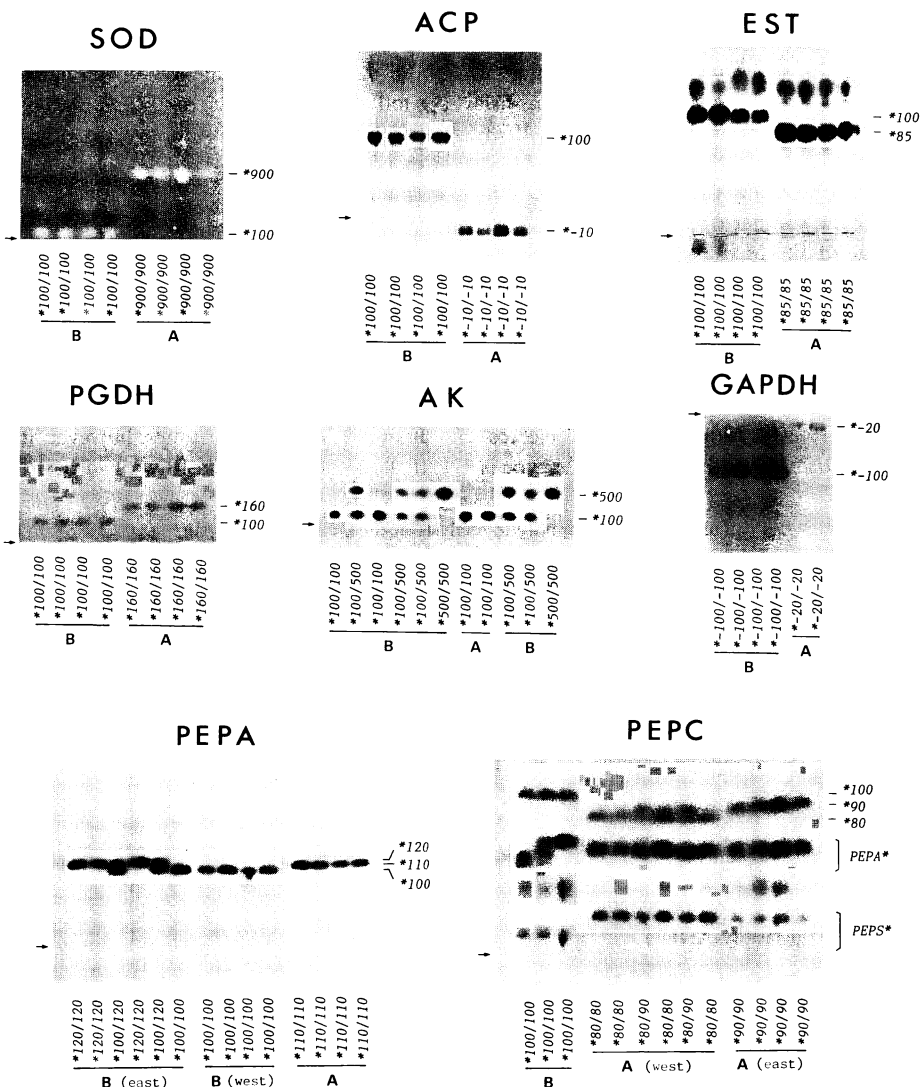
Fig. 2.

many arguments still remain about its former drainage patterns (Yokoyama, 1969). The accumulation of gene frequency data from further collections in this area may help explain the geological events.

Among the populations of Type B, genetic variation at the Ak^* locus was observed in specific rivers. In future, other genetically different populations associated with geographical events may be found.

The estimated divergence time based on the examined D values between Types A and B is 3 million years ago according to Nei's (1975) protein calibrations. At this time, it is believed that a freshwater area was broadly located in the present Seto Inland Sea area, the so-called Second Seto Lake, and that vigorous speciation of freshwater fishes occurred there (Ichihara, 1966; Tsubokawa, 1988). The present distribution of Type A is mainly along the Seto Inland Sea and Lake Biwa. Type B is probably related to the Korean fishes. There is a good possibility that Type A is derived from Type B, which had extended its range from Korea to the Second Seto Lake.

Recently, several calibrations have been proposed to estimate divergence times from genetic distance data (Nei, 1987). It is considered that there is no molecular clock which is universal for all organisms.
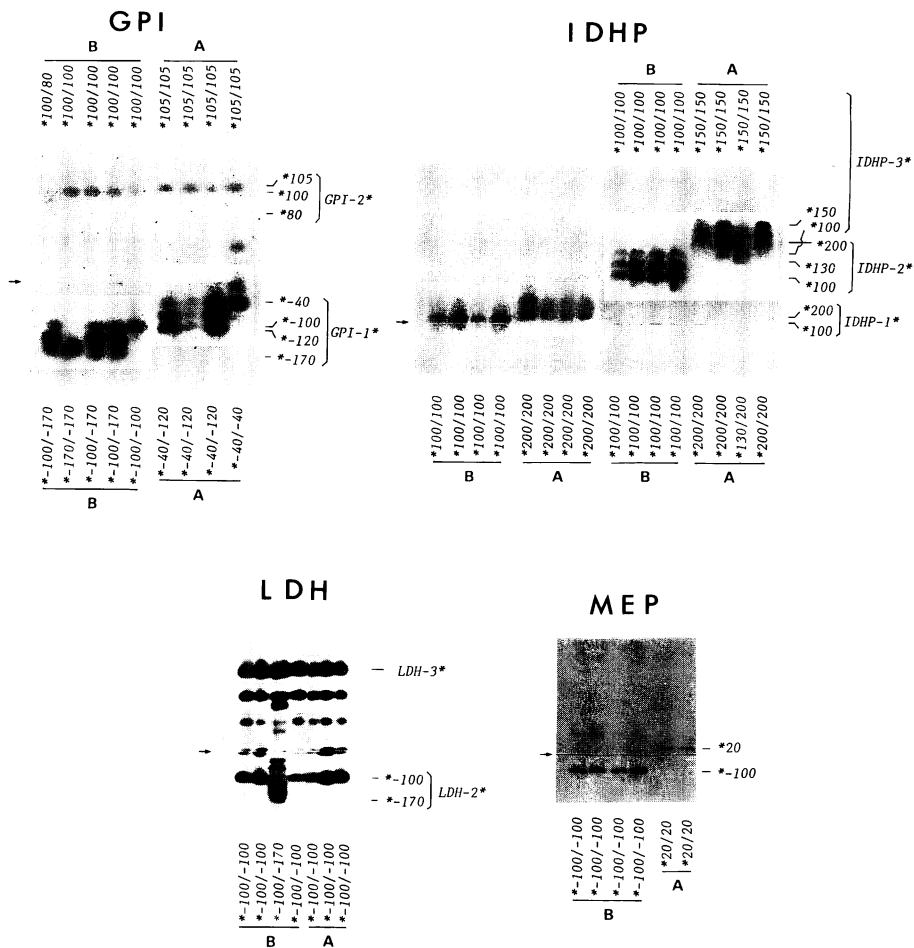
Fig. 2. Isozyme patterns of dark chub. SOD = superoxide dismutase, ACP = acid phosphatase, EST = esterase, PGDH = phosphogluconate dehydrogenase, AK = adenylate kinase, GAPDH = glyceraldehyde-3-phosphate dehydrogenase, PEPA = dipeptidase, PEPC = peptidase-c, GPI = glucose-6-phosphate isomerase, IDHP = isocitrate dehydrogenase, LDH = l-lactate dehydrogenase, MEP = malic enzyme (NADP⁺). Alleles are indicated at the right of each gel. Scoring of individual genotypes is indicated at the top and bottom of each gel. An arrow indicates the sample origin; anode is toward the top. A: A type fish. B: B type fish. Note that the PEPS* locus was not routinely scored.

Accordingly, we have to set up a standard molecular clock for cyprinid fishes using fossil records in these districts.

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Table 2. Observed allelic frequencies at diagnostic and polymorphic loci from 17 dark chub populations in Japan. Sample numbers correspond to location numbers in Fig. 1.

Location	Sample size	ACP*		AK*		EST*		GAPDH*		GPI-1*					GPI-2*			IDHP-1*	
		*100	*−10	*100	*500	*100	*85	*−100	*−20	*−100	*−20	*−170	*−120	*−40	*100	*80	*105	*100	*200
Type B																			
1. Shinbori R.	15	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000
3. Ibi R.	18	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000
4. Suzuka R.	15	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000
5. Ano R.	30	1.000	0.000	0.769	0.231	1.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000
6. Kumozu R.	13	1.000	0.000	0.864	0.136	1.000	0.000	1.000	0.000	0.962	0.000	0.038	0.000	0.000	1.000	0.000	0.000	1.000	0.000
7. Tokida R.	23	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	0.978	0.000	0.022	0.000	0.000	1.000	0.000	0.000	1.000	0.000
8. Ane R.	10	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	0.950	0.000	0.050	0.000	0.000	1.000	0.000	0.000	1.000	0.000
9. Yasu R.	7	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000
11. Asahi R.	19	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	0.895	0.053	0.053	0.000	0.000	0.921	0.079	0.000	1.000	0.000
Total	150	1.000	0.000	0.946	0.054	1.000	0.000	1.000	0.000	0.970	0.023	0.007	0.000	0.000	0.990	0.010	0.000	1.000	0.000
Type A																			
2. Nagara R.	3	0.000	1.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	1.000	0.000	1.000
4. Suzuka R.	4	0.000	1.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	1.000	0.000	1.000
5. Ano R.	1	0.000	1.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	1.000	0.000	1.000
6. Kumozu R.	6	0.000	1.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000	0.833	0.167	0.000	0.000	1.000	0.000	1.000
7. Tokida R.	10	0.000	1.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000	0.600	0.400	0.000	0.000	1.000	0.000	1.000
8. Ane R.	10	0.000	1.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	1.000	0.000	1.000
10. Yasu R.	4	0.000	1.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	1.000	0.000	1.000
11. Asahi R.	5	0.000	1.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	1.000	0.000	1.000
Total	43	0.000	1.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000	0.884	0.116	0.000	0.000	1.000	0.000	1.000

Table 2. (Continued)

Location	IDHP-2*			IDHP-3*		LDH-2*			MEP*		PEPA*			PEPC*			PGDH*		SOD*	
	*100	*200	*130	*100	*150	*−100	*−10	*−170	*−100	*20	*100	*120	*110	*100	*80	*90	*100	*160	*100	*900
Type B																				
1. Shinbori R.	1.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000
3. Ibi R.	1.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	1.000	0.000	0.250	0.750	0.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000
4. Suzuka R.	1.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000
5. Ano R.	1.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000
6. Kumozu R.	1.000	0.000	0.000	1.000	0.000	0.955	0.000	0.045	1.000	0.000	0.038	0.962	0.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000
7. Tokida R.	1.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000
8. Ane R.	1.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000
9. Yasu R.	1.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000
11. Asahi R.	1.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000
Total	1.000	0.000	0.000	1.000	0.000	0.996	0.000	0.004	1.000	0.000	0.273	0.727	0.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000
Type A																				
2. Nagara R.	0.000	1.000	0.000	0.000	1.000	1.000	0.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	1.000
4. Suzuka R.	0.000	0.875	0.125	0.000	1.000	1.000	0.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	1.000
5. Ano R.	0.000	1.000	0.000	0.000	1.000	1.000	0.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	1.000
6. Kumozu R.	0.000	0.700	0.300	0.000	1.000	1.000	0.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	1.000
7. Tokida R.	0.000	1.000	0.000	0.000	1.000	1.000	0.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	1.000
8. Ane R.	0.000	0.900	0.100	0.000	1.000	0.900	0.100	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.850	0.150	0.000	1.000	0.000	1.000
10. Yasu R.	0.000	1.000	0.000	0.000	1.000	1.000	0.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	1.000	0.000	1.000
11. Asahi R.	0.000	0.800	0.200	0.000	1.000	1.000	0.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.900	0.100	0.000	1.000	0.000	1.000
Total	0.000	0.902	0.098	0.000	1.000	0.976	0.024	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.289	0.711	0.000	1.000	0.000	1.000

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遺伝的に識別されるカワムツ (*Zacco temmincki*) の2型について

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背鰭と胸鰭の色彩の違いによって識別されるカワムツ (*Zacco temmincki*) の2型 (A・B) における遺伝的分化の程度をアイソザイムを用いて検討した．10水系で採集した個体に基づいて分析した結果，両者の間には検討した27遺伝子座のうち13に遺伝子の置換が認められた．両型が同所的に分布している水系においても雑種とみられる個体は認められないことから，カワムツのA型及びB型は別種として扱うことが妥当と判断された．

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