Loss of Duplicated Gene Expression in Japanese Char, Salvelinus pluvius

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Loss of Duplicated Gene Expression in Japanese Char, *Salvelinus pluvius*

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Summary

Twenty four isozyme systems of cultured Japanese char (*Salvelinus pluvius*) were examined by starch gel electrophoresis for the existence of duplicated genes. The existence of duplicated gene loci were found for sixteen isozyme sytems and they indicated a disomic mode of inheritance. Assuming that the duplicated gene was lost in 8 isozyme systems, the proportion of the duplicated gene loci was estimated at 66.7%. It seems more likely that loss of duplicate gene expression will occur quite rapidly after the tetraploid event in Japanese char.

Three of eight cultured populations indicated a null allele polymorphism at one locus of duplicate muscle-specific isocitrate dehydrogenase loci. The significance of the finding is that loss of duplicate gene expression is still occurring in the salmonids and that approximately 50% of the original duplicate gene expression has been lost.

Polyploidy has been implicated in the evolution of salmonid fishes. The ancestor of the salmonids fishes is thought to have undergone a tetraploid event some 50-100 million years ago (1, 2). Cytogenetic evidence suggests that the salmonid fishes are derived from an autotetraploid (3). In a newly arisen autotetraploid, the chromosomes should pair as quadrivalents during meiosis and then subequent diploidzation by chromosomal rearrengements (centric fusions, translocation, inversion, etc.) will reestablish bivalent pairing in autotetraploids to varying degrees (1). The extensive karyotypic variation between and within various salmonid species reflects such ancestral chromosomal rearrangements (3).

The electrophoretic patterns of salmonid fishes indicate extensive gene duplication, suggesting that the salmonid fishes are derived from an autotetraploid. Allendorf *et al.* (2) reported that apporximately 50% of the structual gene loci detected by electrophoresis are duplicated in rainbow trout. Fujio and Imura (4) reported that the proportion of duplicated genes was high (54% to 86%) in eight salmonid fishes. Kijima and Fujio (5) estimated the proportion of the duplicated gene loci in chum salmon to be 80%. Therefore, salmonid fishes have lost about 20% to 50% of the original duplicated gene expression.

Duplicated gene expression is thought to have been lost by fixation of null alleles which either does not produce a protein or produces a protein with greatly reduced activity at structural or regulatory loci (1, 6). In the process, disomic inheritance must first be reestablished, because the lethality was caused by a null allele segregating at a tetrasomic locus. Null alleles are difficult to detect in a single locus except through the absence of activity in the homozygote. In duplicated gene loci, however, null alleles must be considered in isozyme studies because of the tendency to identify heterozygotes with a null allele as a homozygote for the active allele which is present. Therefore, null allele polymorphisms would be one indication that loss of duplicated gene expression is still occurring. Null allele polymorphisms have been found in a few of salmonid fishes. For example, null alleles were reported at an isocitrate dehydrogenase locus, a malic enzyme locus, a phosphoglucomutase locus, and an aspartate aminotransferase locus in brook trout, at a lactate dehydrogenase locus in rainbow trout, and at a lactate dehydrogenase, a malate dehydrogenase and creatine phosphokinase locus in brown trout. In the event, null allele polymorphisms are generally assumed to be rare (7).

In this work, we describe the duplicated gene loci for the isozymes of Japanese char (*Salvelinus pluvius*) and present evidence that unusual phenotypic distributions of the muscle-specific, duplicated isocotrate dehydrogenase (m-IDH) loci in the cultured populations are due to a null allele polymorphism. The loss of duplicated gene expression in a diploid-tetraploid relationship of salmonid species shall be discussed.

Materials and Methods

Eight cultured populations of Japanese char (*Salvelinus pluvius*) were sampled from several hatcheries for electrophoresis (Table 1). Several tissues, mainly skeletal muscle and liver, were taken from each individual, immediately stored and kept at −80°C until tested. For electrophoresis, the clear supernatant was

TABLE 1. *Collection Informations of Japanese char*

Population	Origin	Date	No. of fish	Mean of body length(mm)
Fukushima-1	Tochigi	1980. 4/26	10	28.40
Fukushima-2	Fukushima	1980. 4/26	19	20.06
Fukushima-3	Iwate	1980. 4/26	29	11.95
Nikko	Tochigi	1982. 8/ 2	30	19.60
Kazuno	Aomori	1982. 8/27	30	17.36
Miyagi-1	Miyagi	1982. 7/26	42	15.11
Miyagi-2	Miyagi	1983. 5/31	54	24.44
Miyagi-3	Miyagi	1983. 5/31	91	14.96

prepared by centrifugation at 3,500 rpm for 30 min. after grinding tissue with equal volumes of distilled water in glass homogenizers.

Electrophoresis was carried out on horizontal starch gels in tris-citrate buffer system (8). The gel buffer was 13.5 mM tris and 4.3 mM citrate (ph 7.0) for the usual analysis and was one half of the concentration for the muscle-specific isocitrate dehydrogenase (m-IDH). The electrode buffer was 0.135 M tris and 0.043 M citrate (pH 7.0). The gels, $200 \times 140 \times 6$ mm, were run at a constant voltage of 300 V for 4 or 6 hrs. at 4°C. For the detection of enzymes, gels were stained according to Shaw and Prasad (9). Superoide dismutase (SOD) was detected by modifying the method of Ayala *et al.* (10) and aspartate aminotransferase (AAT) by the method of Johnson and Utter (11).

Relative density of isozymes was obtained scanning the stained gel with a recording densitometer. The relative ratios of isozymes produced were calculated by measuring the area bounded by the densitrometric curves.

Results

1. Duplicated isozyme loci

The enzymes, alcohol dehydrogenase (ADH), aspartate aminotransferase (AAT), α-glycerophoshate dehydrogenase (αGPD), glucosephosphate isomerase (GPI), isocitrate dehydrogenase (IDH), lactate dehydrogenase (LDH), malate dehydrogenase (MDH), malic enzyme (ME), 6-phosphogluconate dehydrogenase (6PGD), phosphoglucomutase (PGM), sorbitol dehydrogenase (SDH), and superoxide dismutase (SOD) were examined for the existence of duplicated loci.

ADH : Three bands were observed in skeletal muscle, one of them migrating slowly toward the cathode and the other two bands toward the anode. The band migrating toward the cathode was expressed predominantly in liver (Fig. 1A). The three bands were interpreted as homodimers of A1 and A2 subunits with one heterodimer between them, indicating a dimeric structure of the enzyme. The fixed pattern was further interpreted as two disomic loci (*Adh-A1* and *Adh-A2*), indicating the gene duplication.

AAT : AAT exhibited three bands in liver and two bands in heart and skeletal muscle (Fig. 1B). The former could be interpreted as a soluble form (s-AAT) and the latter as a mitochondial form (m-AAT). The s-AAT was expressed as homodimers of B1 and B2 subunits with one heterodimer between them, ,indicating a dimeric structure. It indicates that s-AAT is controlled by two disomic loci, *Aat-B1* and *Aat-B2*, wich are monomorphic. The m-AAT was also expressed as homodimers of A1 and A2 subunits with no heterodimer between them. This again indicates that m-AAT is controlled by two disomic loci, *Aat-A1* and *Aat-A2*, which are monomorphic. Thus, in both forms of AAT designated as *A* and *B* loci gene duplication was revealed.

αGPD : The six bands migrating toward the anode were observed in heart

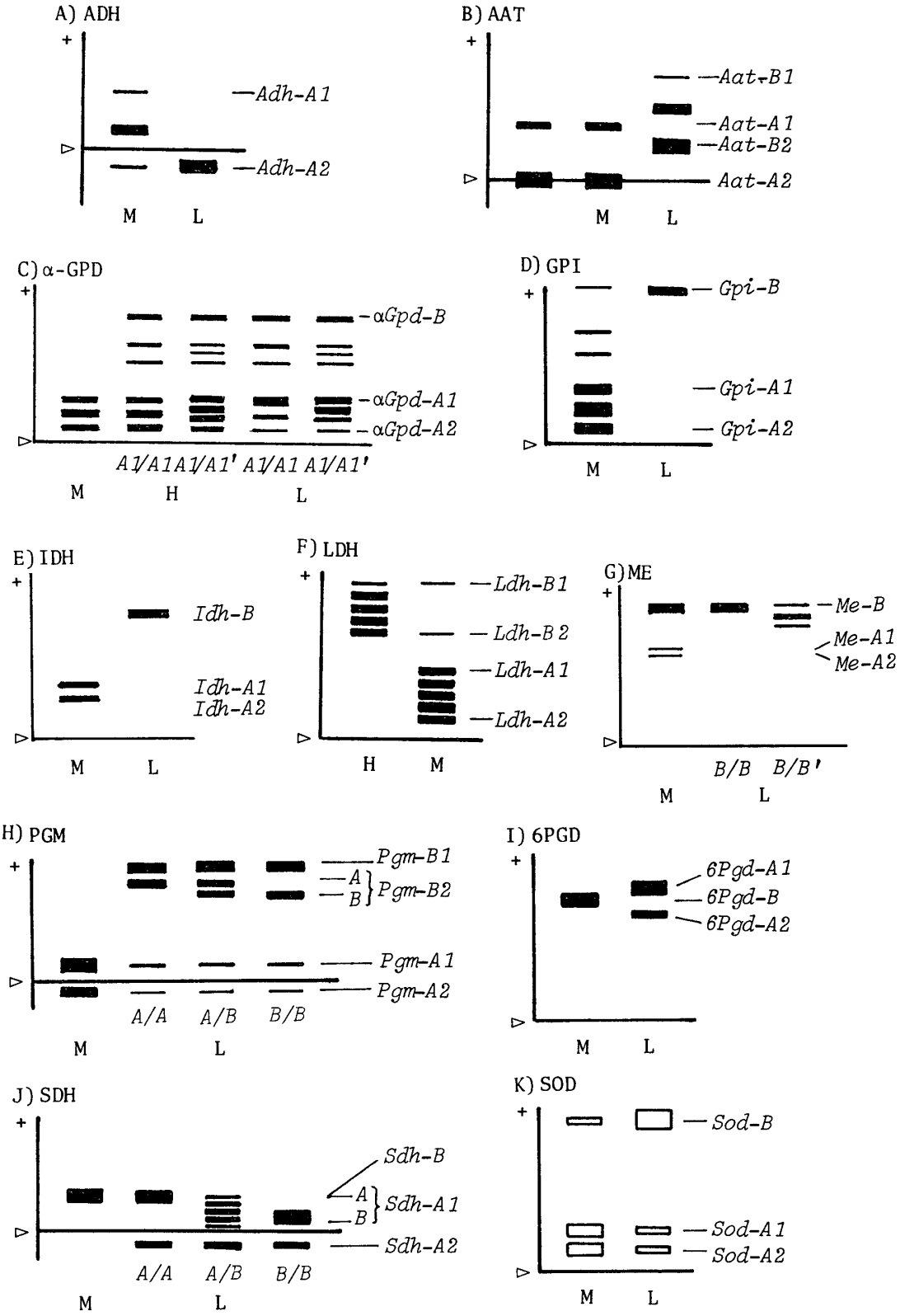

FIG. 1. Summary of the isozyme patterns examined in Japanese char. H, M, and L indicate heart, skeletal muscle, and liver, respectively.

and liver, and the slower three bands were observed in skeletal muscle. The six bands were characterized by three homodimer of A1, A2, and B and three heterodimers of B with A1 and A2 and between A1 and A2 subunits on the basis of a dimeric structure. On skeletal muscle, variants were observed in two of the eight populations. They showed five bands in heterozygotes (*A1/A1′*), resulting from the association of A1 whth A1′ subunit and of A2 with A1 and A1′ subunits. The homodimer of A1′ was located in an identical electrophoretic position with the heterodimer between A1 and A2. The variants were in very low frequency and the homozygote (*A1′/A1′*) was not observed (Fig. 1C). Thus, αGPD was characterized by two separate loci, *A* and *B*, and the gene duplication of *A* locus was revealed but of *B* locus could not be demonstrated.

GPI : GPI pattern showed a six-banded phenotype. In most fish, the GPI is three-banded, indicating a dimeric structure with separate gene loci, *A* and *B*, which code for A and B subunits. The A subunit is strongly predominant in skeletal muscle, while the B subunit is expressed lightly in skeletal muscle but strongly in liver. A similar expression was observed in Japanese char (Fig. 1D). The fixed bands were characterized by three homodimers of A1, A2, and B, and three heterodimers of B with A1 and A2, and between A1 and A2 subunits. Thus, GPI was characterized by two separate loci, *A* and *B*, and the gene duplication of *A* locus was revealed but of *B* locus could not be demonstrated.

IDH : IDH isozymes were shown to posses a dimeric structure and observed to consist of a soluble form (s-IDH) in liver and a mitochondrial form (m-IDH) in skeletal muscle, as shown in chum salmon (5). The m-IDH was shown as the fixed two bands except in three of the eight populations (Fig. 1E). Two bands resulted from homodimers of A1 and A2 subunits with no heterodimer between them. Thus, it could be interpreted as two loci, *Idh-A1* and *Idh-A2*. The s-IDH was shown as a fixed single band migrating the fastest toward the anode in liver and could be interpreted as one locus, *Idh-B*. Thus, the existence of gene duplication could not be demonstrated.

LDH : The pattern of Japanese char showed the typical multi-banded phenotype observed in the salmonid fishes like chum salmon. The fast migrating five bands were observed in the heart and the slow migrating five bands in skeletal muscle (Fig. 1F). The separate gene loci, *A1* and *A2*, were discovered in five molecular forms in skeletal muscle and two separate gene loci, *B1* and *B2*, were found in the heart.

MDH : MDH consists of soluble forms (s-MDH) and mitochondrial forms (m-MDH). Three distinct bands were observed in s-MDH of skeletal muscle in Japanese char. The homodimeric isozyme of A subunit migrated more slowly toward the anode than did the homodimeric isozyme of B subunit. The homodimeric isozyme of B subunit was predominantly found in skeletal muscle (s-MDH-B) while the homodimeric isozyme of A subunit was predominantly

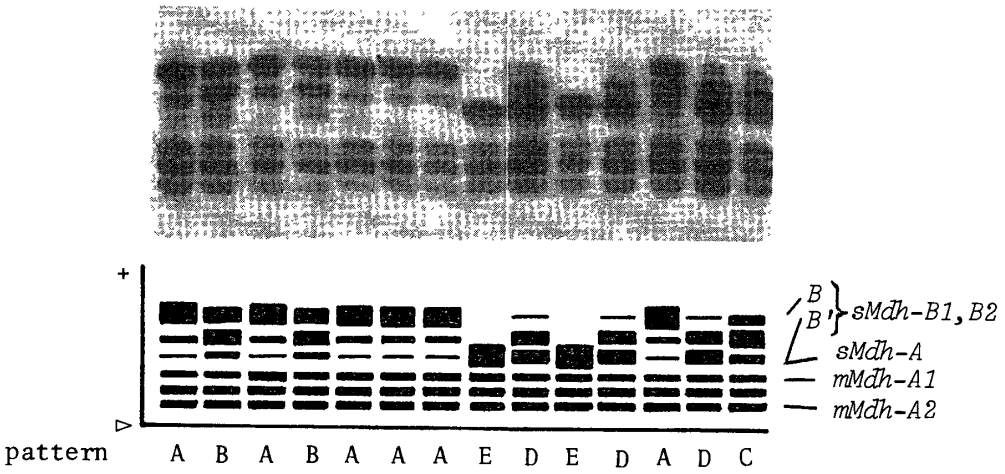

FIG. 2. Electrophoretic patterns of MDH in skeletal muscle of Japanese char. A, B, C, D, and E indicate five phenotypes.

found in heart (s-MDH-A). The m-MDH exhibited three fixed bands migrating most slowly toward the anode. The extensive survey of s-MDH from 294 specimens in eight populations revealed variant pattern and most of the patterns were classified into five types, A, B, C, D, and E type (Fig. 2). The most prevalent A type exhibited three equally spaced bands with staining intensity strongest in the most anodal band, followed by the second and then third band. The B, C, and D types showed three bands in identical electrophoretic positions with the A type, while the E type showed a single band in an identical positions with the third band. In the B type, the second third bands stained more intensely than those of the A type. In the C type, the second band stained more intensely than the most anodal one. In the D type, the staining intensity was lightest in the most anodal band, followed by the second and then the third band. Since a homodimeric isozyme of A subunit always appeared with the same lightly-staining band in all phenotypes, it was presumed that the B, C, D, and E types were the variants of B subunit (B′). The homodimeric isozyme of B′ subunit appeared in an identical position with the homodimeric isozyme of A subunit. If the MDH-B is controlled by a single disomic locus which codes for the B subunit, the A, C, and E types will be interpreted but the B and D types can not be interpreted. Thus, these variant phenotypes suggest the gene duplication of *B* locus. The other variant phenotypes were observed in three populations, Fukushima-2, Fukushima-3, and Kazuno. These phenotypes are presumed to be the variants of B subunit. There are marked differences among eight populations (Table 2).

ME : ME was examined in skeletal muscle and liver and activity was exhibited in two zones (Fig. 1G). In the slow anodal zone, two bands were observed in skeletal muscle of all indiviiuals. The two fixed bands could be interpreted as

TABLE 2. *Phenotype and Gene Frequency of s-MDH of Japanese char*

Population	Pattern							Frequency		
	A	B	C	D	E	others	total	q*B*	q*B′*	others
Fukushima-1	6	3	1	—	—	—	ft	0.875	0.125	0
Fukushima-2	—	2	3	8	2	4	19	0.355	0.553	0.092
Fukushima-3	1	15	5	1	—	7	29	0.647	0.275	0.078
Nikko	24	5	1	—	—	—	30	0.942	0.058	0
Kazuno	4	9	9	2	—	6	30	0.658	0.283	0.059
Miyagi-1	—	2	8	16	5	—	31	0.306	0.694	0
Miyagi-2	1	3	19	18	13	—	54	0.319	0.681	0
Miyagi-3	0	7	29	33	22	—	91	0.308	0.692	0

two disomic loci, *Me-A1* and *Me-A2*. In the most anodal zone, a single band was observed in liver and muscle of almost all individuals examined (Me-B). Variants were observed in two of the eight populations. They showed the three banded heterozygotes (*B/B′*) consisting of the homodimers of B and B′ subunits and the heterodimer between them. However, variants were found in very low frequency and the homozygote (*B′/B′*) was not observed. The existence of duplicated gene at the *Me-A* locus was not domonstrated.

PGM: PGM was examined in skeletal muscle and liver of Miyagi-3 population, and total number of four bands were observed in the two zones. In the first zone (PGM-A), two bands were observed in skeletal muscle. One of the bands migrated slower toward the anode and the other toward the cathode. The two fixed bands could be interpreted by two disomic loci, *Pgm-A1* and *Pgm-A2*, which code for A1 and A2 subunits, respectively. In the second zone (PGM-B), two bands migrating fastest toward the anode were predominant in liver. The two bands could be interpreted by two disomic loci, *Pgm-B1* and *Pgm-B2*. In

TABLE 3. *Genotype and Gene Frequency of Pgm-B2 and Sdh-A1 of Japanese char*

Population	Genotype				Frequency		
	A/A	*A/B*	*B/B*	total	*qA*	*qB*	χ^2
Pgm-B2							
Miyagi-2	16(17.2)	29(26.6)	9(10.2)	54	0.565	0.435	0.441
Miyagi-3	21(17.8)	34(40.4)	26(22.8)	81	0.469	0.531	2.038
Sdh-A1							
Miyagi-2	25(22.7)	20(24.6)	9(6.7)	54	0.648	0.352	1.882
Miyagi-3	36(37.4)	38(35.3)	7(8.3)	81	0.679	0.321	0.463

Number in parenthesis represents the expected number under the Hardy-Weinberg's equilibrium.

TABLE 4. *Summary of the Isozyme Loci examined in Japanese char*

Enzyme	Tissue specificity	Locus	Variation	Duplication
ADH	skeletal muscle and liver	*Adh-A1*	−	+
		Adh-A2	−	
m-AAT	heart and skeletal muscle	*Aat-A1*	−	+
		Aat-A2	−	
s-AAT	liver	*Aat-B1*	−	+
		Aat-B2	−	
α-GPD	skeletal muscle	*αGpd-A1*	+	+
		αGpd-A2	−	
	liver	*αGpd-B*	−	−
GPI	skeletal muscle	*Gpi-A1*	−	+
		Fpi-A2	−	
	liver	*Gpi-B*	−	−
m-IDH	skeletal muscle	*Idh-A1*	+	+
		Idh-A2	−	
s-IDH	liver	*Idh-B*	−	−
LDH	skeletal muscle	*Ldh-A1*	−	+
		Ldh-A2	−	
	heart and liver	*Ldh-B1*	−	+
		Ldh-B2	−	
s-MDH	heart	*sMdh-A*	−	−
	skeletal muscle	*sMdh-B1*	+	+
		sMdh-B2	+	
m-MDH	skeletal muscle	*mMdh-A1*	−	+
		mMdh-A2	−	
ME	skeletal muscle	*Me-A1*	−	+
		Me-A2	−	
	liver	*Me-B*	+	−
PGM	skeletal muscle	*Pgm-A1*	−	+
		Pgm-A2	−	
	liver	*Pgm-B1*	−	+
		Pgm-B2	+	
6PGD	liver	*6Pgd-A1*	−	+
		6Pgd-A2	−	
	skeletal muscle	*6Pgd-D*	−	−
SDH	liver	*Sdh-A1*	+	+
		Sdh-A2	−	
	skeletal muscle	*Sdh-B*	−	−
SOD	skeletal muscle	*Sod-A1*	−	+
		Sod-A2	−	
	liver	*Sod-B*	−	−

the PGM-B, three distinct phenotypes, designated A, AB, and B, were observed (Fig. 1H). Thus, *Pgm-B1* locus was monomorphic while *Pgm-B2* locus was polymorphic (Table 3).

6PGD : 6PGD was examined in skeletal muscle and liver of Miyagi-3 population, and three bands were observed in the anodal side. Two fixed bands were observed in liver and a single fixed band in skeletal muscle (Fig. 1I). Thus, the existence of duplicated gene was demonstrated in liver (6Pgd-A) but not in skeletal muscle (6Pgd-B).

SDH : SDH was examined in skeletal muscle and liver of Miyagi-3 population. Two bands were observed in liver , one of them migrating toward the anode and the other toward the cathode (Fig. 1J). A single cathodal band always appeared in all individuals while in the anodal zone three phenotypes, A, AB, and B, were observed. These three distinct phenotypes are the variants of *A1* locus (*B*). This indicates that the liver SDH is controlled by two disomic loci, *Sdh-A1* and *Sdh-A2*. *Sdh-A1* locus is polymorphic (Table 3) while *Sdh-A2* locus is monomorphic. A single band was observed in skeletal musle of all individuals and the band was located in an identical position with the homodimer of A1 subunit in liver.

SOD : SOD exhibited three fixed anodal bands in skeletal muscle of all individuals. The two fixed bands in the slow anodal zone could be interpreted as two disomic gene loci, *Sod-A1* and *Sod-A2*, as in *Me-A*. A single band in the most anodal zone was observed in liver and muscle, as in *Me-B* (Fig. 1K).

The results are summarized in Table 4. The isozyme systems indicated the gene duplication to a disomic mode of inheritance. Assuming a duplicated gene loss in *αGpd-B*, *Gpi-B*, *Idh-B*, *sMdh-A*, *Me-B*, *6Pgd-B*, *Sdh-B*, and *Sod-B*, the proportion of duplicated gene loci was estimated as 66.7% in Japanese char.

2. *A null allele polymorphism*

The extensive survey of m-IDH revealed variant patterns in three of the eight populations. In Miyagi-1, Miyagi-2, and Miyagi-3 populations which originated

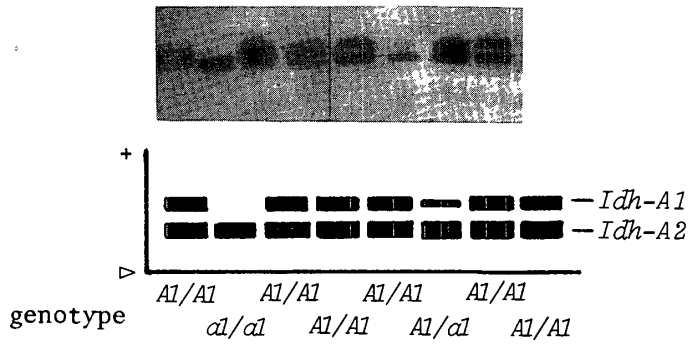

FIG. 3. Electrophoretic patterns of m-IDH in skeletal muscle of Japanese char.

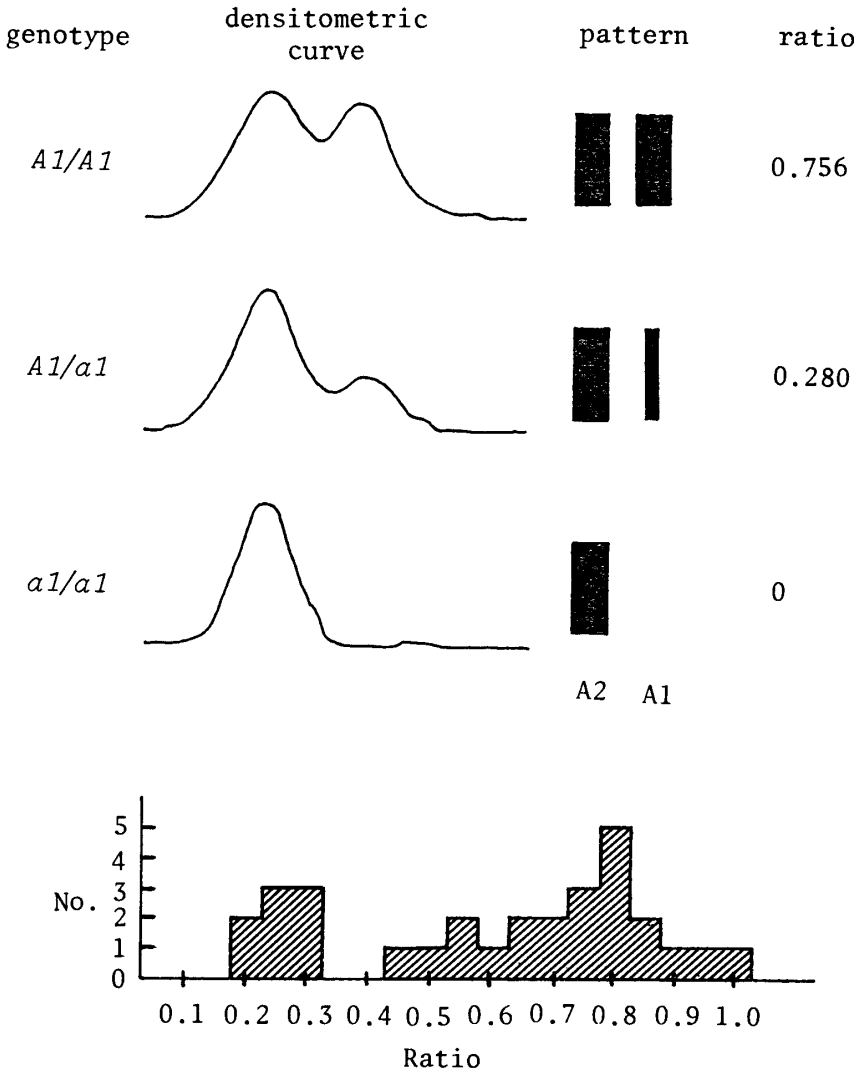

FIG. 4. Densitrometrical tracings of the three phenotypes of the m-IDH isozymes in skletal muscle of Japanese char. Ratios are Al isozyme to A2 isozyme in densitometrical densities.

from Kurikoma, three distinct phenotypes were found as shown in Fig. 3. In normal type, m-IDH was exhibited as two bands in skeletal muscle. The two variant types were two banded and a single banded, respectively. Two bands of the former type showed identical positions with normal type and the second band stained more intensely than more anodal one. A single band of the latter showed identical position with the second band. These phenotypes indicate that the recessive null mutant (*a1*) is present in relatively high frquency in Miyagi populations. The homozygote appeared as a single band in the ubiquitous (A2) system.

We propose the *A1/A1*, *A1/a1*, and *a1/a1* genotypes for the phenotypes. The ratios of A1 isozyme produced to the A2 isozyme were densitometrically

TABLE 5. *Genotype and Gene Frequency of Idh-A1 of Japanese char*

Population	Genotype				Frequency		
	Al/Al	*Al/al*	*al/al*	total	*qAl*	*qal*	χ^2
Fukushima-1	10	—	—	10	1.000	0	—
Fukushima-2	19	—	—	19	1.000	0	—
Fukushima-3	29	—	—	29	1.000	0	—
Nikko	30	—	—	30	1.000	0	—
Kazuno	30	—	—	30	1.000	0	—
Miyagi-1	33(32.6)	8(8.8)	1(0.6)	42	0.881	0.119	0.344
Miyagi-2	44(43.5)	9(9.9)	1(0.6)	54	0.898	0.102	0,354
Miyagi-3	74(74.2)	6(5.7)	0(0.1)	80	0.963	0.037	0.116

Number in parenthesis represents the expected number under the Hardy-Weinberg's equilibrium.

measured. The result is shown in Fig. 4. Heterozygote (*A1/a1*) was indicated in relatively lower activity compared to homozygote (*A1/A1*), suggesting the production of subunits with depended on the gene dose. Furthermore, the phenotypic distributions of Idh-A1 isozyme loci were not significant in Chi-square tests for goodness of fit to Hardy-Weinberg's proportions (Table 5).

Discussion

The present work estimated the proportion of the duplicated gene loci in Japanese char to be 66.7%. Allendorf *et al.* (2) reported that 8 of the 16 isozymic loci were duplicated gene loci in rainbow trout. Fujio and Imura (4) estimated the proportion of duplicated gene loci in 13 isozymic systems of sockeye salmon, chum salmon, biwa salmon, rainbow trout, steelhead trout, brown trout, Japanese char, and brook trout, and the obtained values were 53.8, 61.5, 53.8, 61.5, 53.8, 69.3, and 84.6%, respectively. Kijima and Fujio (5) reported that the proportion of the duplicated gene loci was estimated as 80.0% from 20 isozymic systems in chum salmon. The difference of both values in chum salmon was due to the demonstration of gene duplication when polymorphism could be found in the case of a fixed single banded phenotype. The differences of the proportion of duplicated gene loci among salmonid species suggest that the rate of duplicate gene expression is different among salmonid species. In this connection, Ferris *et al.* (12) described that the speciation event was a factor influencing the rate of loss of duplicate gene expression. Li (13) concluded that a slow rate of chromosomal diploidinzation was the major factor influencing post-tetraploid evolution of salmonids.

The present work revealed the existence of null allele polymorphism at the banding pattern that could distingish between duplicate loci with a different mobility for the second locus. The existence of a null allele polymorphism at

clearly disomic loci have been reported in Salvelinus and Salmo genus. Stoneking *et al.* (14) found a null allele at an isocitrate dehydrogenase, malic enzyme, and phosphoglucomutase loci in brook trout. These interpretations were based solely on the electrophoretic banding pattern. Furthermore, Stoneking *et al.* (14) proposed a null allele polymorphism as an aspartate aminotrasferase locus for the aberrant phenotypic distributions found in wild brook trout populations. Allendorf *et al.* (15) described variants in brown trout at lactate dehydrogenase and a creatine phosphokinase locus that could be interpreted as either mobility variants or null alleles. A null allele has been documented at a lactate dehydrogenase locus in rainbow trout (16) and malate dehydrogenase locus in brown trout (17).

The existence of a null allele polymorphism is of interest and importance in the process of gene loss at the duplicate loci. In the process of gene loss, time is divided into three stages. The first one is from tetraploidization to reestablishment of disomic segregation of chromosomes. During this period, gene loss can occur only through loss of chromosomes. Such loss has probably rarely occurred in salmonids, because the number of chromosome arms and the DNA content seem to remain at the tetraploid level (1). Persistence of tetrasomic segregation will greatly retard the rate of gene loss. In the second stage after the reestablishment of disomic segregation, loss of duplicate gene expression has no deleterious effect because the other locus has two normal genes. As time proceeds, the third stage inters when regulatory or functional divergence of duplicate genes proceeds to a substantial extent. It is assumed that one locus is more expressed than the other, at least in one tissue. Such phenomenon was observed as differences in activity between two isozymes coded by duplicate gene loci in Japanese char. For example, the activity of A1 isozyme was higher than A2 isozyme in muscle-specific phosphoglucomutase, and the activity of B2 was higher than B1 isozyme in the liver-specific isozyme. Furthermore, in the *A1/A1* homozygote of *Idh-A1* locus, the activity of A1 isozyme was weaker than the A2 isozyme coded by the other locus. The behavior of several isozyme systems exhibits a disomic mode of inheritance. No evidence of tetrasomic inheritance is indicated in Japanese char (this paper) and chum salmon (5). It is assumed that salmonids are at a stage after the reestablishment of disomic segregation.

The evidence of a muscle-specific isocitrate dehydrogenase null allele, along with other null allele polymorphisms in salmonids, conclude that loss of duplicate gene expression is still occuring.

Acknowledgements

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