Duplicated Isozyme Loci in Chum Salmon

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Akihiro KIJIMA and Yoshihisa FUJIO

Department of Fishery Science, Faculty of Agriculture,
Tohoku University, Sendai, Japan

Summary

Twenty isozyme systems of chum salmon (*Oncorhynchus keta*) were examined by starch gel electrophoresis in order to elucidate the existence of duplicated genes. The results can be classified into three groups: 1) The existence of the duplicated gene was demonstrated in a fixed multi-banded phenotype. Such isozyme systems were LDH-A, LDH-B, LDH-E, α-GPD-A, α-GPD-B, s-IDH, SDH, ME-A, ME-B, s-AAT, m-AAT, PGM-A and SOD, and they indicate the disomic mode of inheritance. 2) A fixed single-banded phenotype could not indicate the existence of gene duplication without a polymorphism. Such isozyme systems were m-MDH, s-MDH-A, PGM-C and 6-PGD. This case may be interpreted by the alternative hypothesis that the duplicated gene was lost or the expression occurred in identical electrophoretical position with original gene. 3) When a polymorphism was found in the case of a single-banded phenotype, the existence of the duplicated gene was demonstrated and the genetic variants appeared to segregate disomically. Such isozyme systems were s-MDH-B, m-IDH and PGM-B.

Assuming that the duplicated gene was lost in m-MDH, s-MDH-A, PGM-C and 6-PGD, the proportion of the duplicated gene loci was estimated at 80.0%. It seems more likely that the genes coding for isozyme systems are inherited disomically in chum salmon. The conclusion is that chum salmon are originally a tetraploid species in the process of diploidization.

Salmonid fish have been demonstrated to be in a diploid-tetraploid relationship to each other with respect to the DNA contents, the number of chromosome arms, and the number of gene loci of lactate dehydrogenase (LDH) isozyme (1, 2). The hypothesis postulates that the salmonid fish have decended from a tetraploid ancestor which had been derived by tetraploidization from a diploid primitive chorade. Many analyses of individual gene loci for isozymes have been performed to ascertain the diploid-tetraploid relationship in rainbow trout (2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16). In rainbow trout, the duplicated genes have been demonstrated for lactate dehydrogenase (LDH), malate dehydrogenase (MDH), α-glycerophosphate dehydrogenase (α-GPD), sorbitol dehydrogenase (SDH), isocitrate dehydrogenase (IDH) and aspartate aminotransferase (AAT), but not for phosphoglucomutase (PGM), superoxide dismutase (SOD), alcohol dehydrogenase (ADH), esterase (ES) and malic enzyme (ME). The gene duplication has also

been demonstrated for some isozyme systems of king salmon, sockeye salmon, Atlantic salmon, brown trout, brook trout, lake trout and splake trout (3, 4, 6, 7, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26). The duplicated genes of chum salmon have been reported for only three isozyme systems, LDH, MDH and AAT.

Fujio and Imura (27) estimated the proportion of duplicated gene loci in 13 enzymic loci coding for 10 enzymes 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, 53.8, 69.3 and 84.6%, respectively.

Numachi et al. (9) investigated the polymorphism of the B subunit of MDH in rainbow trout and concluded the subunit to befit the tetrasomic mode of inheritance. They (9) also reported the B subunit of MDH in chum salmon to befit a similar tetrasomic inheritance. Wolf et al. (14) suggested a tetrasomic inheritance for the s-IDH gene in rainbow trout. Ropers et al. (16) observed that the polymorphism of the s-IDH indicated a tetrasomic mode of inheritance in rainbow trout and they performed the mating experiments in order to test whether this mechanism was responsible for phenotype distribution. They concluded that in the majority of matings, the phenotype could be interpreted by the genetic mode of two disomic loci. However in other tetrasomic loci, segregation was more likely to have occurred.

On the other hand, Allendorf et al. (13, 15) concluded on breeding experiments of rainbow trout that IDH was controlled by two disomic loci. The existence of gene duplication for IDH isozymes of chum salmon was reported previously by Kijima and Fujio (28), who suggested that the disomic inheritance from an asymmetrical distribution of isozyme produces in the variant phenotypes.

The purposes of the present work are to demonstrate the duplicated gene loci for the isozymes of chum salmon and to elucidate the mode of inheritance.

Materials and Methods

Specimens of chum salmon, Oncorhynchus keta, were supplied from salmon hatcheries located in Hokkaido in 1978, for electrophoretical analysis. Several tissues, mainly skeletal muscle and liver were taken from each individual, immediately frozen with dry-ice, and stored at $-80°C$ until tested.

For the usual analyses of the phenotypic distribution, the cell-lysate obtained by freezing and thawing was directly subjected to electrophoresis. For the densitometrical measurements, the clear supernatant was prepared by centrifugation at 10,000 r.p.m. for 30 min. after grinding tissues with equal volumes of distilled water in glass homogenizers. Starch gel electrophoresis was carried out by the procedures previously reported (28). Superoxide dismutase (SOD) was detected by modifying the method of Ayala et al. (29) and aspartate aminotransferase (AAT) by the method of Johnson and Utter (30). The other enzymes were detected by

modifying the methods complied by Shaw and Prasad (31).

Relative density of isozymes was obtained by scanning the stained starch gel with the Jookoo densitometer, Densitoron model-pan. The relative ratios of isozymes produced were calculated by weighing the paper which was cut along the densitometric tracings.

Results

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

LDH: LDH pattern of chum salmon showed the typical multi-banded phenotype observed in the salmonid fishes as rainbow trout. LDH is the tetrameric structure of the enzyme and the separate gene loci, *A* and *B*, which code for A and B subunits. In most fish, the A subunit was expressed in skeletal muscle while the B subunit was strongly predominant in heart. In chum salmon, the fast migrating five LDH isozymes were observed in the heart and the slow migrating five isozymes in skeletal muscles. This resulted in complete loss of the affinity between A and B subunits, and no additional LDH bands appeared in the space between the two series. Thus, separate gene loci, *A1* and *A2*, were discovered in five molecular forms in skeletal muscle and two separate genes, *B1* and *B2*, were found in the heart.

Additional five isozymes, migrating the fastest toward the anode, were observed in the eye. The five fixed bands could be interpreted by two disomic loci, as well as LDH-A and LDH-B. Thus, the LDH observed in the eye were directed by two duplicated loci, *Ldh-E1* and *Ldh-E2*, which coded for E1 and E2 subunits involving five molecular forms.

Genetic variation was not observed in heart and eye. Three distinct phenotypes were observed in skeletal muscle. Since the homotetramer of A2 subunit always appeared in all phenotypes, three phenotypes indicated two alleles, *Ldh-A1^A* and *Ldh-A1^B* at the *Ldh-A1* locus.

α-GPD: The three bands migrating the fastest toward the anode were observed in liver (α-GPD-B). These three bands resulted in the forms of two homodimers of B1 and B2 subunits and one heterodimer between them, indicating a dimeric structure of the enzyme. The other two bands were observed in skeletal muscle (α-GPD-A), and one of them migrated slowly toward the anode and the other toward the cathode. The two bands resulted in the forms of two homodimers of A1 and A2 subunits and lacked a heterodimer between them. Thus, the α-GPD was directed to two separate loci, *A* and *B*, and the gene duplication of each locus was revealed. The four loci coding for α-GPD were monomorphic.

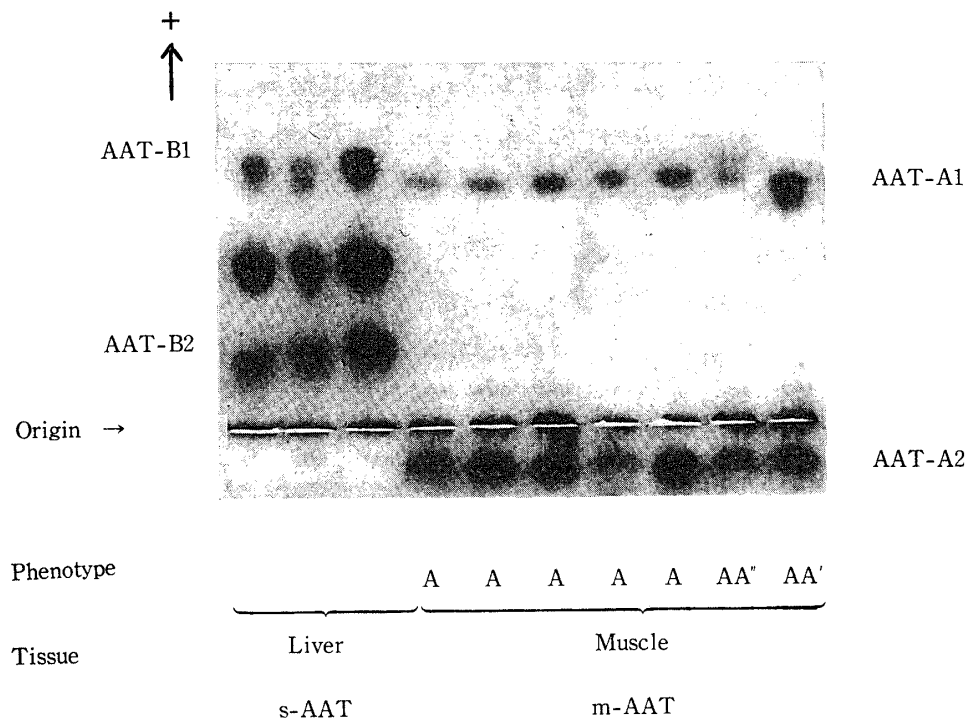AAT: AAT exhibited three bands in liver and two bands in skeletal muscle

Fig. 1.   Electrophoretical patterns of AAT in liver and skeletal muscle of chum salmon.
A, AA′ and AA″ indicate three phenotypes of AAT-Al isozyme.

as shown in Fig. 1.   The former could be interpreted as a soluble form (s-AAT) and the latter as a mitochondrial form (m-AAT).   The s-AAT resulted in the forms of two homodimers of B1 and B2 subunits and one heterodimer between them, indicating a dimeric structure of the enzyme.   Thus, it indicated that s-AAT was controlled by two disomic loci, *Aat-B1* and *Aat-B2*, which were monomorphic.

In m-AAT, a cathodal single band always appeared in all phenotypes and the anodal bands corresponded with A, AA′ and AA″ type.   The m-AAT resulted in the forms of two homodimers of A1 and A2 subunits and lacked a heterodimer between them.   These three distinct phenotypes are the variants of *A1* locus (*A1′* and *A1″*).   The three bands exhibited in heterozygotes (*A1/A1′* and *A1/A1″*), indicated a probable dimeric structure of the enzyme.   In other words, the AA′ and AA″ types resulted from the association of A1 subunit with A1′ and A1″ subunits, respectively.   The variants were very low in frequency in chum salmon populations and the homodimer of each A1′ and A1″ subunit was not observed.   This indicates that m-AAT is controlled by two disomic loci, *Aat-A1* and *Aat-A2*.   The *Aat-A1* locus was polymorphic and *Aat-A2* locus was monomorphic.

ME: ME was exmained in heart and skeletal muscle and liver, and the ME activity was exhibited in two zones.   In the most anodal zone, two bands in total number were observed in all tissues.   Although two bands were predominant in liver, one of them migrated faster and was predominant in heart, and the other one was predominant in skeletal muscle.   The two fixed bands could be interpreted as

two disomic loci, *Me-A1* and *Me-A2*. In the second zone, five bands were observed in heart and skeletal muscle of all individuals. The five bands resulted in the forms of two homomers of B1 and B2 subunits and three heteromers between them, indicating a tetrameric structure of the enzyme. Thus, the fixed five bands could be interpreted as two disomic loci, *Me-B1* and *Me-B2*. The four loci coding for ME were monomorphic.

SOD: SOD exhibited two fixed anodal bands in skeletal muscle of all individuals. The two fixed bands could be interpreted as two disomic gene loci, as well as ME-A.

SDH: SDH exhibited three fixed anodal bands in liver and resulted in the forms of two homodimers of separate subunits and one heterodimer between them. The fixed three bands could be interpreted as two disomic loci.

IDH: IDH isozymes were a dimeric structure and were observed to consist of a soluble form (s-IDH) in liver and a mitochondrial form (m-IDH) in skeletal muscle, as reported previously (28). The s-IDH isozymes were controlled by two disomic loci, *A1* and *A2*. The *A1* locus was monomorphic while the *A2* locus was polymorphic. The *A2* locus indicated five alleles, $A2^A$, $A2^B$, $A2^C$, $A2^D$ and $A2^E$.

The three distinct phenotypes of m-IDH, designated F, M and S, were observed as shown in Fig. 2. These three phenotypes were directed by two disomic loci, *B1* and *B2*, which coded the B1 and B2 subunits. The slowest migrating isozyme was the variant of B2 subunit (B2′). The two bands of S type indicated that homodimers of B1 and B2′ subunits were produced in the ratio of *a*:*b* (Table 1). The ratio of *a*:*b* was 1:1.9 in densitometrical measurements of the S type isozymes, that is, the ratio of B1 and B2 subunits produced was almost 1:2 (Fig. 3). This different production between subunits indicates the control of different loci. The pattern of M type indicated three bands were formed by random association of two subunits under the control of two alleles, *B2* and *B2′*, in the heterozygotes.
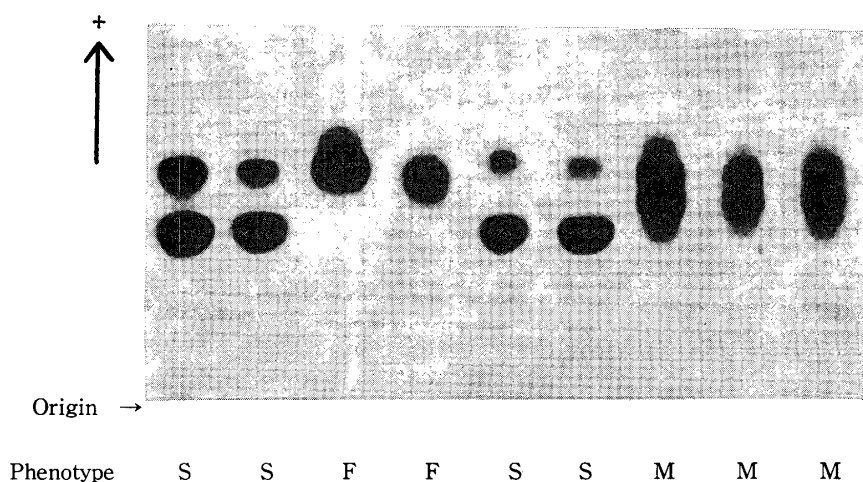

FIG. 2. Electrophoretical patterns of m-IDH in skeletal muscle of chum salmon. F, M and S indicate three phenotypes.

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TABLE 1.   *Postulated Genotypes of m-IDH Isozyme and Theoretical*
Ratio of Isozymes Produced in Each Genotype

Phenotype	Genotype		Ratios of isozyme composed of		
	locus *B1*	*B2*	B1B1+B2B2	B2B2′	B2′B2′
F	*B1B1*	*B2B2*	$a+b$	—	—
M	*B1B1*	*B2B2′*	$a+1/4\ b$	$1/2\ b$	$1/4\ b$
S	*B1B1*	*B2′B2′*	a	—	b

Ratios of isozymes composed of

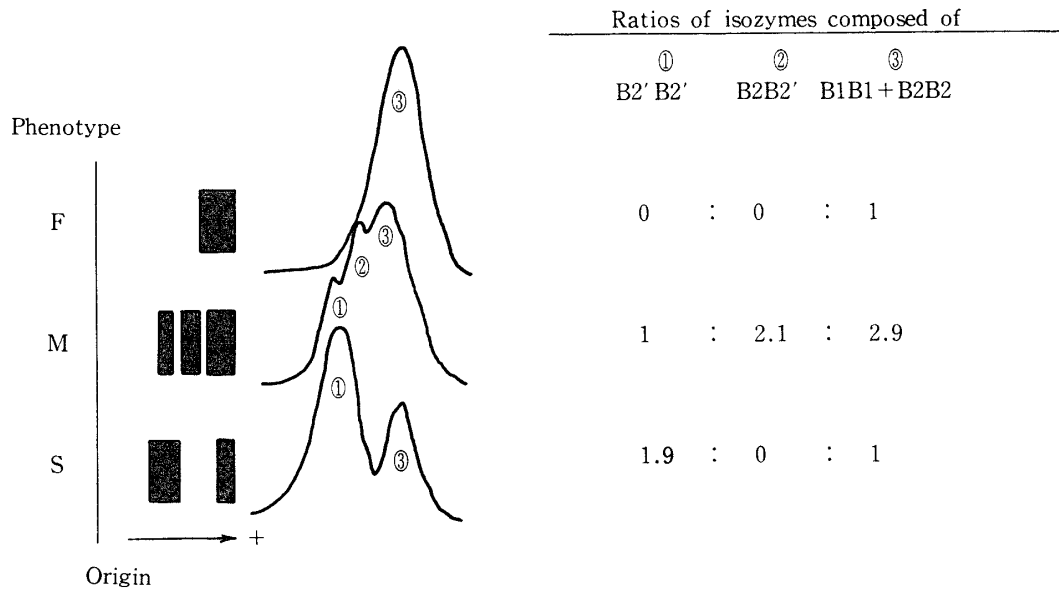|	①	②	③
Phenotype	B2′B2′	B2B2′	B1B1+B2B2
F	0	: 0	: 1
M	1	: 2.1	: 2.9
S	1.9	: 0	: 1

FIG. 3.   Densitometrical tracings of the three phenotypes of the m-IDH isozymes in
skeletal muscle of chum salmon.

Since the B1 subunit isozyme shows an identical electrophoretical position with
the B2 subunit isozyme, the ratio of isozymes produced in them is theoretically
given by $(a+1/4\ b)$: $1/2\ b$: $1/4\ b$. Assuming that the ratio of a: b is 1:2, the
ratio of isozymes produced in M type is estimated as 3:2:1. The ratio of isozymes
produced in M type was 2.9:2.1:1 in densitometrical measurements as shown in Fig.
3, that is, the ratio was almost 3:2:1. Thus, it confirms that m-IDH is controlled
by two disomic loci, *B1* and *B2*. This is the case of a single-banded phenotype in
most individuals of a population, and the existence of a duplicated gene is
indicated when a polymorphism is observed.

PGM: PGM was exmained in heart and skeletal muscle and liver, and four
bands in total number were observed in the three 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 coded for A1 and
A2 subunits, respectively. In the second zone (PGM-B), one band was observed
in heart and skeletal muscle. In the last zone (PGM-C), one band migrating the

fastest toward the anode was predominant in liver. PGM-C showed a fixed single-banded phenotype in all individuals of a population. The existence of a duplicated gene could not be demonstrated.

In PGM-B, three distinct phenotypes, designated F, M and S, were observed (Fig. 4). The F type exhibited an intensely-staining band migrating toward the anode. The M type exhibited two bands, one of which showed an identical electrophoretical position with F type isozyme and stained intensely, and the other migrated slowly and stained lightly. The two bands in the S type showed an identical electrophoretical positions with M type and were identical in staining properties. If the PGM-B is directed by a single disomic locus, the two isozymes produced in M type could not be interpreted. If the PGM-B is under a single tetrasomic locus, the reverse type of intensely-staining in two bands would be observed. Such phenotype was not observed.

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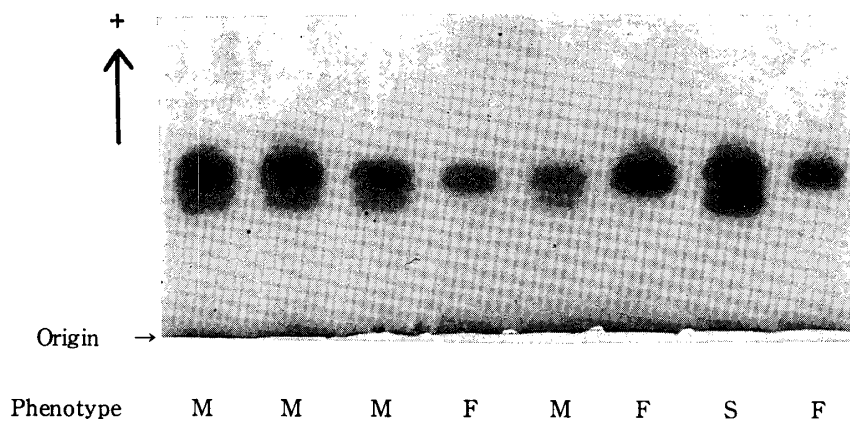Origin →

| Phenotype | M | M | M | F | M | F | S | F |

FIG. 4. Electrophoretical patterns of PGM-B in skeletal muscle of chum salmon. F, M and S indicate three phenotypes.

Assuming that the PGM phenotypes are directed by two disomic loci, which code for B1 and B2 subunits, and the slowest migrating isozyme is the variant of B2 subunit (B2′), a similar interpretation with m-IDH can be given for the three phenotypes of this enzyme. The pattern of S type indicates two B1 and B2′ subunits separately, and the ratio of isozymes produced is given by $a:b$ (Table 2). The ratio of $a:b$ was 1.0:1 in densitometrical measurements of the S type (Fig. 5). The patterns of M type indicated two isozymes formed by B1 and B2′ subunits under the control of two alleles in the heterozygotes, indicating a monomeric structure of the enzyme. Since the B2 subunit isozyme shows an identical electrophoretical position with the B1 subunit isozyme, the ratio of isozyme produced in M type is theoretically given by $(a+1/2\ b): 1/2\ b$ (Table 2). Assuming that the ratio of $a:b$ is 1:1, the ratio of $(a+1/2\ b): 1/2\ b$ is estimated as 3:1. The ratio obtained in denistometrical measurements of M type was 2.9:1 (Fig. 5). The agreement
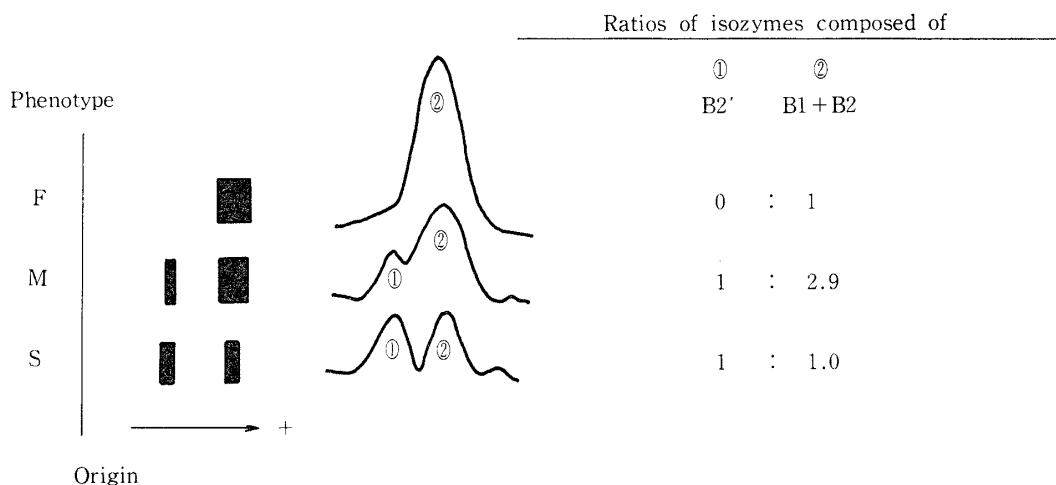
Fig. 5. Densitometrical tracings of the three phenotypes of the PGM-B isozymes in skeletal muscle of chum salmon.

Table 2. *Postulated Genotypes of PGM-B Isozyme and Theoretical Ratio of Isozymes Produced in Each Genotype*

Phenotype	Genotype		Ratios of isozymes composed of	
	locus *B1*	*B2*	B1+B2	B2'
F	*B1B1*	*B2B2*	$a+b$	—
M	*B1B1*	*B2B2'*	$a+1/2\ b$	$1/2\ b$
S	*B1B1*	*B2'B2'*	a	b

of the obtained ratio with theoretical ratio indicates that the PGM-B is controlled by two disomic loci.

The distribution of PGM-B phenotypes was examined in the chum salmon populations of the Chitose, Kushiro and Nishibetsu rivers (Table 3). The phenotypic frequencies were in better agreement with a Hardy-Weinberg equilibrium of $(p+q)^2$ under two disomic loci than that of $(p+q)^4$ under a tetrasomic locus in all populations. Thus, this confirms that the PGM-B is controlled by two disomic loci, *Pgm-B1* and *Pgm-B2*. *Pgm-B1* locus was monomorphic and *Pgm-B2* locus was polymorphic.

MDH: MDH consists of soluble forms (s-MDH) and mitochondrial forms (m-MDH). In general, s-MDH is a dimeric molecule and two separate gene loci code for A and B subunits of this enzyme. The A and B subunits associate at random and form three dimeric forms, which can be visualized as three distinct bands.

A similar pattern was observed in s-MDH of skeletal muscle in chum salmon. 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), and the homodimeric

TABLE 3 *Observed Numbers of Phenotypes of PGM-B Isozymes and Expected Numbers for Each Type in Disomic or Tetrasomic Inheritance*

Posulated genotypes	Observed number of phenotypes					χ^2
	F	M	S			
disomic	B1B1B2B2	B1B1B2B2'	B1B1B2'B2'			
tetrasomic	BBBB	BBBB'	BBB'B'	BB'B'B'	B'B'B'B'	
Chitose	16 $\left(\begin{matrix}17.1\\22.6\end{matrix}\right)$	41 $\left(\begin{matrix}38.8\\32.8\end{matrix}\right)$	21 $\left(\begin{matrix}22.1\\17.9\end{matrix}\right)$	— $\left(\begin{matrix}-\\4.3\end{matrix}\right)$	— $\left(\begin{matrix}-\\0.4\end{matrix}\right)$	0.2503 9.2143
Kushiro	13 $\left(\begin{matrix}12.4\\17.1\end{matrix}\right)$	30 $\left(\begin{matrix}31.1\\26.4\end{matrix}\right)$	20 $\left(\begin{matrix}19.5\\15.2\end{matrix}\right)$	— $\left(\begin{matrix}-\\3.9\end{matrix}\right)$	— $\left(\begin{matrix}-\\0.4\end{matrix}\right)$	0.0808 7.2897
Nishibetsu	9 $\left(\begin{matrix}8.9\\14.0\end{matrix}\right)$	29 $\left(\begin{matrix}29.2\\25.3\end{matrix}\right)$	24 $\left(\begin{matrix}23.9\\17.0\end{matrix}\right)$	— $\left(\begin{matrix}-\\5.1\end{matrix}\right)$	— $\left(\begin{matrix}-\\0.6\end{matrix}\right)$	0.0029 10.9092

Number above and under in parenthesis represents the expected numbers for each type according to a Hardy-Weinberg equilibrium of $(p+q)^2$ and $(p+q)^4$, respectively.

isozyme of A subunit was predominantly found in heart (s-MDH-A). The m-MDH exhibited a fixed single band migrating most slowly toward the anode.

The extended survey of s-MDH from 927 specimens revealed the variant patterns and all of their patterns were classified into four types, A, B, C and D type (Fig. 6). The most prevalent A type exhibited three equally spaced bands, while the B and C type exhibited four bands and the D type five bands. Three of the four bands in B and C types showed identical electrophoretical positions with A type, and the remaining band appeared between the homodimeric isozyme of B subunit and the heterodimeric isozyme of A and B subunits. In the B type, the staining intensity was the strongest in the most anodal band, followed by the second and then third band. In the C type, the second band stained more intensely than the most anodal one. In the D type, three of five bands showed an identical electrophoretical positions with the A type, and the remaining two bands migrated faster than the most anodal band of the A type. 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 and D types were the variants of B subunit (B' and B''). In the B and C types, the homodimeric isozyme of B' subunit appeared extremely near in position to the heterodimeric isozyme between A and B. Also, in the D type, the heterodimeric isozyme between B'' and A subunits appeared extremely near in position to the homodimeric isozyme of B.

If the MDH-B is directed by a single disomic locus which codes the B subunit, the ratio of BB: BB' isozyme produced in the B type or the ratio of B''B'':BB'' in the D type will be theoretically given as 1:2. Since the distribution of isozymes produced in the B and D type was not in agreement with the theoretical ratio, the
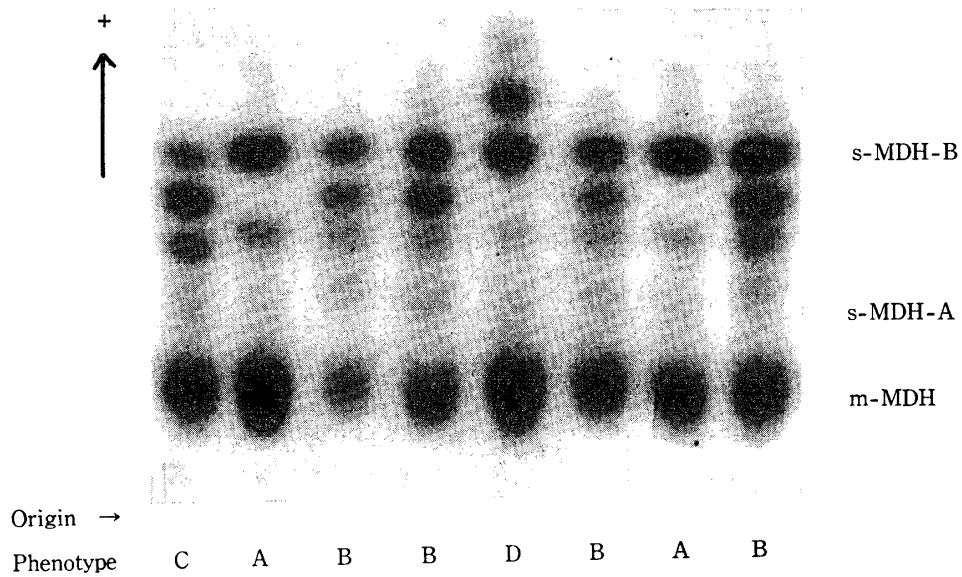
FIG. 6. Electrophoretical patterns of s-MDH in skeletal muscle of chum salmon. A, B,
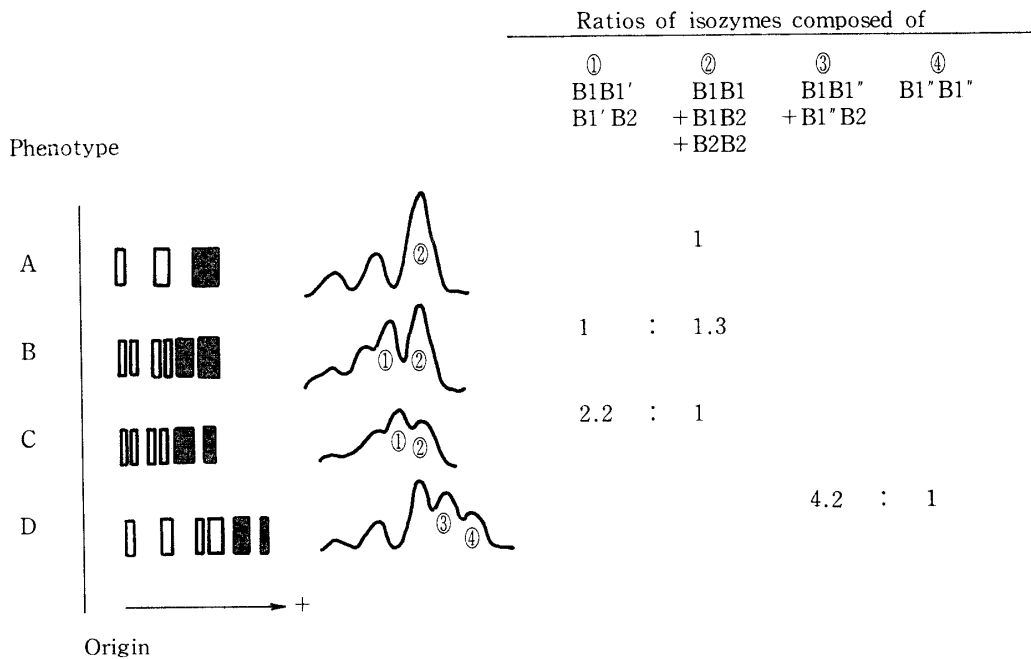C and D indicate four phenotypes.

FIG. 7. Densitometrical tracings of the four phenotypes of the s-MDH-B isozymes in
skeletal muscle of chum salmon.

MDH-B could not be explained under a single disomic locus. If the MDH-B is
directed by a single tetrasomic locus, the ratio of BB:BB′ in the B type will be 9:6,
the ratio of BB:BB′ in the C type will be 1:2, and the ratio of B″B″:BB″ in the
D type will be 1:6. The ratio of BB:BB′ in the B type was 1.3:1 (7.8:6), and the
ratio in the D type was 1:4.2 in densitometrical measurements (Fig. 7). The

obtained ratios were distinctly different from the theoretical ratios. This indicates that the MDH-B can not be explained under a single tetrasomic locus.

Assuming that the MDH-B is directed by two disomic loci, the four phenotypes can be explained. If the B2 subunit isozyme shows an identical electrophoretical position with B1 subunit isozyme, the ratio of $BB:BB':B'B'$ in the B and C type and $BB:BB'':B''B''$ in the D type represent $(B1B1+B1B2+B2B2):(B1B1'+B2B1'):$ $B1'B1'$ and $(B1B1+B1B2+B2B2):(B1B1''+B2B1''): B1''B1''$, respectively. These ratios are theoretically given by $(1/4\ a^2+ab+b^2): (1/2\ a^2+ab):1/4\ a^2$ in the B type, $b^2:2ab:a^2$ in the C type, and $(1/4\ a^2+ab+b^2): (1/2\ a^2+ab): 1/4\ a^2$ in the D type, where a and b represent the ratio of B1 and B2 subunits produced, respectively (Table 4). The ratio of $BB:BB'$ in the B type was 1.3:1 in densitometrical measurements. From the above ratio, it was calculated that the ratio of $a:b$ was 1.3:1. From that the ratio of $BB:BB'$ in the C type of 1:2.2, it was calculated that the ratio of $a:b$ was 1.1:1. From that the ratio of $B''B'': BB''$ in the D type of 1:4.2, it was calculated that the ratio of $a:b$ was 1.8:1. The obtained ratios of $a:b$ were in rought accord with 1.3:1. The densitometrical measurements of MDH-B isozymes showed the different production between B1 and B2 subunits, meaning the control of different loci. Thus, this indicates that the MDH-B isozymes are controlled by two disomic loci rather than a single tetrasomic locus.

TABLE 4. *Postulated Genotypes of MDH-B Isozyme and Theoretical Ratio of Isozymes Produced in Each Genotype*

Phenotype	Genotype		Ratios of isozymes composed of				
	locus *B1*	*B2*	(B1B1+B1B2 +B2B2)	(B1B1' +B1'B2)	B1'B1'	(B1B1'' +B1''B2)	B1''B1''
A	*B1B1*	*B2B2*	$a^2+2ab+b^2$	—	—	—	—
B	*B1B1'*	*B2B2*	$1/4\ a^2+ab+b^2$	$1/2\ a^2+ab$	$1/4\ a^2$	—	—
C	*B1'B1'*	*B2B2*	b^2	$2ab$	a^2	—	—
D	*B1B1''*	*B2B2*	$1/4\ a^2+ab+b^2$	—	—	$1/2\ a^2+ab$	$1/4\ a^2$

6-PGD: 6–PGD showed a fixed single band migrating toward the anode in skeletal muscle of all individuals. Thus, the existence of duplicated gene could not be demonstrated.

From the results, we can summarize the followings: 1) The existence of the gene duplication was demonstrated in the form of a fixed multi-banded phenotype found in all individuals of a population. Such isozyme systems were LDH-A, LDH-B, LDH-E, α-GPD-A, α-GPD-B, s-AAT, m-AAT, ME-A, ME-B, SOD, SDH, s-IDH and PGM-A. 2) The gene duplication could not be demonstrated in the case of a fixed single-banded phenotype found in all individuals of a population. Such isozyme systems were PGM-C, m-MDH, s-MDH-A and 6-PGD. 3) When a polymorphism could be found in the case of a fixed single-banded phenotype, gene

duplication was demonstrable. Such isozyme systems were m-IDH, PGM-B and s-MDH-B.

This concluded that the isozyme systems indicated the duplicated gene to be a disomic mode of inheritance. Assuming that the duplicated gene was loss in PGM-C, m-MDH, s-MDH-A and 6-PGD, the proportion of duplicated gene loci was estimated as 80.0% in chum salmon.

Discussion

The tetrasomic inheritance was reported in hexose-6-phosphate dehydrogenase in brook trout (22), s-isocitrate dehydrogenase (14), sorbitol dehydrogenase (12) and s-malate dehydrogenase (9) in rainbow trout. Numachi *et al.* (9) interpreted the s-MDH-B phenotypes in chum salmon and rainbow trout to be controlled by a single tetrasomic locus. The present work indicated that the s-MDH-B phenotypes were controlled by two disomic loci rather than a single tetrasomic locus, as well as other isozyme systems. There is no evidence for the tetrasomic inheritance but for two disomic inheritance for the duplicated isozyme loci of chum salmon. For these reasons, preferential pairing of the difinite chromosome each of the original four homologies seems to be a probable explanation. The diploidization process in which the ancient tetraploids were involved subsequent to polyploidization suggests that the tetrasomic gene loci become functionally diverse and subject to a disomic mode of inheritance. Thus, the behavior of several isozyme systems confirms the hypothesis that the chum salmon is a tetraploid species in the process of diploidization.

Allendorf (32) reported that the loss or retention of expression by one of duplicated genes was observed with about 50% in rainbow trout. Fujio and Imura (25) reported that the proportion of the duplicated gene was high (54% to 86%) in eight salmonid fish. The present work showed the similar proportion with the assumption that PGM-C, m-MDH, s-MDH-A and 6-PGD were not duplicated in chum salmon. The isozymes in which the existence of a duplicated gene have not been demonstrated for a fixed single-banded phenotype may be interpreted by the following alternative hypothesis. The duplicated gene was lost or retented in the expression, or could not be detected due to the lack of polymorphism.

Polymorphism in *Ldh-A1*, *Mdh-B1*, *Idh-A2*, *Idh-B2*, *Pgm-B2* and *Aat-A1* loci code for each isozyme indicates to be that it will be very useful as genetic markers. These allelic isozymes would be especially advantageous in the analysis of population structure, particularly at the identifications of populations and inbreeding, and the relationships among river population where chum salmon return to spawn their eggs. Determining the effects of the transplanting of hatchery fish on released salmon is a major concern to management. Genetic markers are useful in studying the effect of transplanting hatchery fish among river popula-

tions. In this connection, genetic differences among river populations were reported previously (33).

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