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Genetic Control of Isocitrate Dehydrogenase Isozymes in Chum Salmon

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Summary

NADP-dependent isocitrate dehydrogenase (IDH) isozymes of chum salmon were examined by starch gel electrophoresis. The IDH isozymes in normal type were observed to consist of three bands zone migrating fast to anode and one band zone migrating slowly. The former was soluble form mainly expressed in liver and the latter mitochondrial form a best seen in muscle. Assuming that IDH is a dimeric enzyme structure, the three bands of s-IDH suggest to be formed by two subunits under the control of two duplicate loci. Three phenotypes of s-IDH were demonstrated for 201 specimens of chum salmon captured in two rivers, and the variant showed three slower migrating bands and heterozygotes five bands. It indicates that the s-IDH is controlled by two disomic loci, one of which is polymorphic and the other monomorphic. From the patterns of three phenotypes of m-IDH, it also indicates that the m-IDH is controlled by two disomic loci, one of which is polymorphic and the other monomorphic.

Two different disomic loci for s-IDH were involved in making three molecular forms but two different disomic loci for m-IDH were not. The two m-IDH isozymes under the control of two loci showed an identical electrophoretic position. From the results, the behavior of an enzyme IDH confirms the hypothesis that the chum salmon is originally a tetraploid species in the process of diploidization.

There are some evidences that salmonid fishes have evoluted from a diploid ancestor by tetraploidization during the more recent past. The tetraploidy hypothesis of salmonids was first proposed by Ohno *et al.* (1, 2) who demonstrated that salmonid fishes, such as rainbow trout, brown trout and coho salmon, contain about twice as much DNA per cell and twice as many chromosome arms as the anchovy and other clupeoid fishes. Bailey *et al.* (3) demonstrated the existence of duplicated loci cording for the B subunit of supernatant malate dehydrogenase in king salmon and rainbow trout. Numachi *et al.* (4) and Clayton (5) interpreted polymorphism of the B subunit of this enzyme assuming under the tetrasomic inheritance, based on the asymmetrical distribution of three isozymes.

Stegeman and Goldberg (6) investigated the polymorphism of hexose-6-phosphate dehydrogenase in brook trout and concluded that in one population

this enzyme was inherited tetrasomically while it follows a disomic mode of inheritance in another one. The latter finding was explained by either Robertsonian fusion or loss of duplicated gene. Polymorphism of the s-form of NADP dependent isocitrate dehydrogenase in rainbow trout was reported by Wolf *et al.* (7), who suggested a tetrasomic mode of inheritance for the gene locus of this enzyme. In order to substantiate tetrasomic inheritance for the s-NADP-IDH gene locus in rainbow trout, breeding experiments were performed by Ropers *et al.* (8). They indicated from the results in the majority of mating that the phenotypes can be interpreted by genetic model of two disomic gene loci, A and B rather than a tetrasomic gene locus A for this enzyme. This interpretation is based on the assumption of an identical electrophoretic position of the A and B isozymes.

The purposes of the present work are to demonstrate the polymorphism of NADP dependent isocitrate dehydrogenase (IDH) and the mode of inheritance in chum salmon, and to discuss on the assumption that this species is tetraploid.

Materials and Methods

Specimens of chum salmon, *Oncorhyncus keta*, were supplied from Salmon Hachery of two rivers, Shari and Ukedo river, in 1976. For the analysis of the distribution of IDH phenotypes, 101 and 100 specimens of mature chum salmon captured in Shari river located in Hokkaido and Ukedo river located in Fukushima, respectively, were examined. Whole bodies or respective tissues of fish were immediately frozen with dry ice, and stored at –80°C until tested.

The extracts were prepared by grinding tissues with equal volumes of distilled water in glass homogenizer. The clear supernatant prepared by centrifugation at 3,500 rpm. for 10 min. was directly subjected to electrophoresis. Screening of variants of IDH was mostly carried out on the cell-lysates obtained by freezing and thawing. To detect phenotypes of IDH isozymes, horizontal starch gel electrophoresis was performed on the respective tissue extracts. Electrophoresis was carried out with 11% starch (Connaught Laboratories, Tront, Canada) in 15.5 mM tris and 4.5 mM citrate (pH 7.0). The electrode buffer was 0.155 M tris and 0.045 M citrate (pH 7.0). A voltage of 14.25 V/cm was applied for 6 hours at 4°C. The detection of IDH on the gel was done by specific staining procedure compiled by Show and Prasad (9).

Results

1) *Distribution of IDH in several tissues*

Tissue distribution of IDH in the mature chum salmon was shown in Fig. 1. The total bands appearing in the normal type were four and those bands were divided into two activity zones showing three bands and one band by several tissues. Three faster migrating bands to anode were mainly appeared in liver, indicating

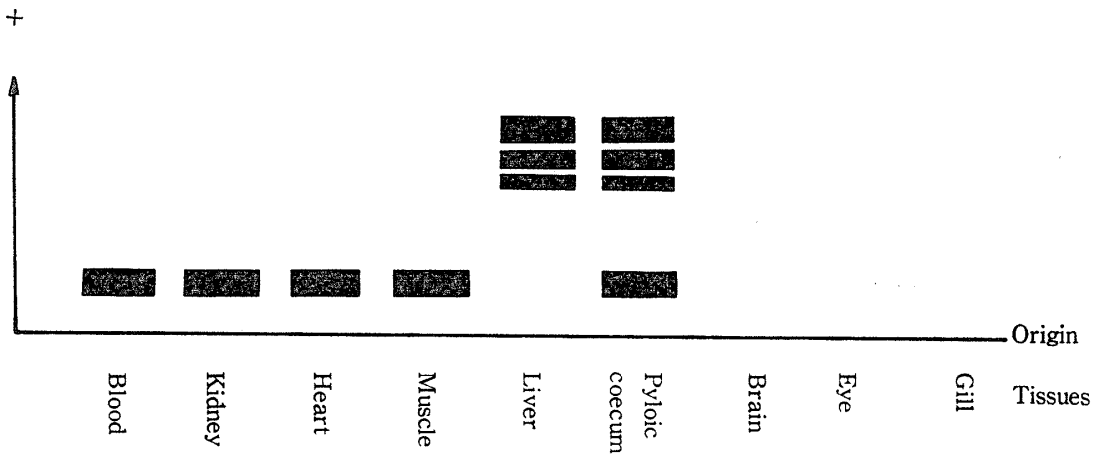

FIG. 1. Electrophoretic patterns of NADP-dependent isocitrate dehydrogenase in several tissues from the chum salmon.

the soluble form (s-IDH). A slower migrating band was observed in heart, kidney, blood, and muscle, indicating the mitochondrial form (m-IDH). Pyloric coecum exhibited both three faster bands and slower migrating one. This observations indicated that no hybrid band between s-IDH and m-IDH was formed. In brain, eye, and gill, activity of IDH was not seen.

The s-IDH was specifically expressed in liver. In the normal type electrophoretic patterns of s-IDH isozymes revealed the occurrence of three isozymes, two kinds of homodimers and one heterodimer of A1 and A2 subunits assuming that the interpretation of IDH phenotypes was based on a dimeric enzyme structure. The homodimeric isozyme of A1 subunit migrated slower than homodimeric isozyme of A2 subunit toward the anodal side.

2) *Genetic control and variant pattern of s-IDH*

Survey of the isozyme patterns of liver from 201 specimens of chum salmon revealed three distinct patterns designated F, M, S types, as shown in Fig. 2. The F and S types exhibited three isozymes, while the M type five isozymes. In the F type, A2 homodimeric isozyme stained the most intensely, and A1 the most lightly. In the S type, the slowest migrating isozyme stained the most intensely and the fastest migrating one stained the most lightly. The fastest migrating isozyme showed an identical electrophoretic position with the A1 isozyme of the F type. Thus, it indicated that the fastest migrating isozyme of the S type was a homodimeric isozyme of A1 subunit, and the slowest migrating isozyme was a variant of A2 subunit (A2′). The third M type exhibited five isozymes, which were composed of F and S type, and an intermediate isozyme showed the most intensely-staining. The intermediate isozyme could be interpreted by an identical electrophoretic position of heterodimeric isozyme of A2 and A2′ with homodimeric isozyme of A1.

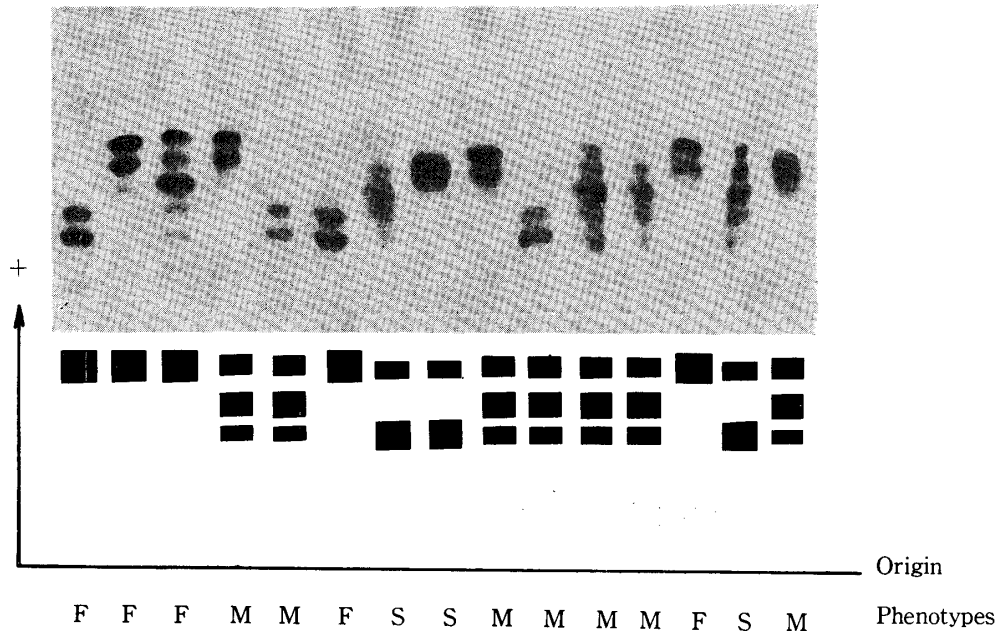

FIG. 2. Three phenotypes of s-IDH and postulated genotypes in liver from chum salmon. F, M, and S indicates the genotype of *A1A1 A2A2*, *A1A1 A2A2'*, and *A1A1 A2'A2'*, respectively.

TABLE 1. *Observed Numbers of Phenotypes of s-IDH and the Gene Frequencies of A2 Locus in the Populations of Chum Salmon.*

Population	Number of tested fish	Phenotype (Observed number)			Frequency of *A2* gene	Chi-square
		F	M	S		
Shari	101	42(42.541)	47(46.015)	12(12.443)	0.649	0.0437
Ukedo	100	32(28.090)	42(49.820)	26(22.090)	0.530	2.4638

Number in parentheses represents the expected numbers for each type according to a Hardy-Weinberg equilibrium of $(p+q)^2$.

The result suggests a genetic model of two dimeric loci, *A1* and *A2*. This pair of gene loci can be considered as equivalent to two alleles of a single locus which codes for the subunit of diploids. The three phenotypes were presumed to be reflected by two alleles, *A2* and *A2'*. Genotype of s-IDH are, therefore, postulated as shown in Fig. 2. Genetic variants were not observed at *A1* locus.

The distribution of phenotypes of A2 subunit isozyme in the populations of chum salmon sampled in Shari and Ukedo river is shown in Table 1. The frequencies of phenotype were in good agreement with the expectation assuming a Hardy-Weinberg equilibrium of $(p+q)^2$. Thus, it indicates that s-IDH is controlled by two disomic loci. Significant difference of gene frequencies between Shari and Ukedo river populations was demonstrated.

3) *Genetic control and variant pattern of m-IDH*

The m-IDH is a best seen in muscle extracts. The isozyme patterns of muscle

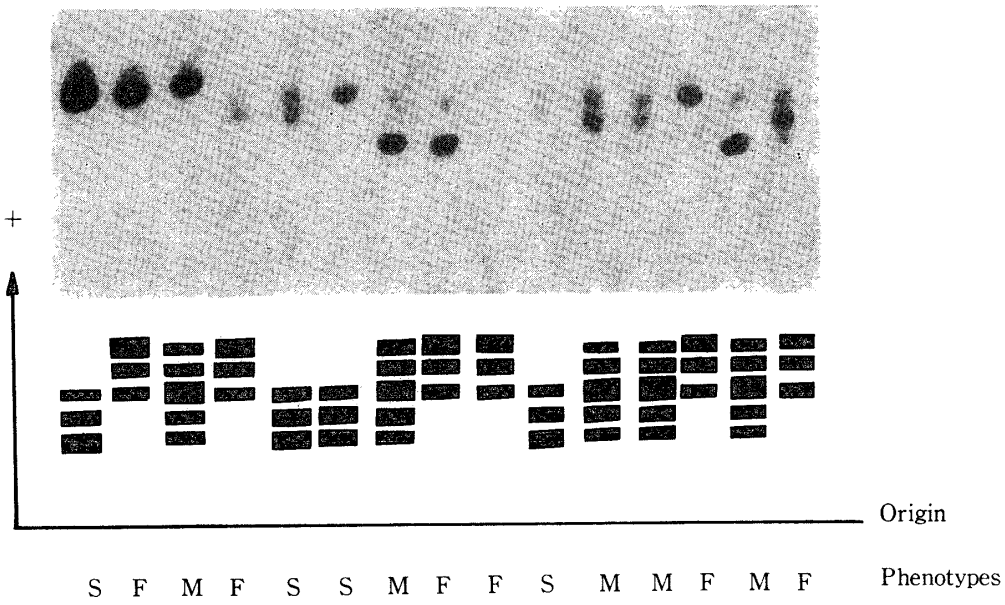

FIG. 3. Three phenotypes of m-IDH and postulated genotypes in muscle from chum salmon. F,M, and S indicates the genotype of *B1B1 B2B2*, *B1B1 B2B2'*, and *B1B1 B2'B2'*, respectively.

revealed three distinct patterns designated F, M, S type (Fig. 3). The most prevalent phenotype was the F type exhibited an intensely-staining band. The S type exhibited two bands, one of them was an identical electrophoretical position with F type isozyme, and the other was slow migrating and intensely-staining one. The S type was observed at low frequency in Shari river population but was not in the Ukedo river population. If the occurence of a faster migrating isozyme in the S type was neglected, the third M type showed three bands which could be considered as hybrid pattern between F and S type. These patterns are, therefore, not in keeping with the genetic model of a single disomic gene locus.

Assuming that m-IDH phenotypes are directed by two disomic loci which code the B1 and B2 subunits and the slowest migrated isozyme is the variant of B2 subunit (B2'), the three phenotypes could be explained. The pattern of M type indicates three isozymes formed by random association of two subunits under the control of two allelic genes in the heterozygotes. The association of two subunits in the heterozygotes would produce three isozymes, B2B2, B2B2', and B2'B2' in the ratio of 1:2:1. The pattern of M type, however, included the asymmetrical distribution of isozymes. Since the B1 subunit isozyme shows an identical electrophoretical position with the B2 subunit isozyme, the ratio of isozymes produced in them is given by the (a B1B1+1/4 b B2B2): (1/2 b B2B2'): (1/4 b B2' B2'), where a and b represent the ratio of B1 and B2 subunits to be produced according to the numbers of B1 and B2 alleles in each genotype as shown in Table 2. The pattern of S type indicates two B1 and B2' subunits separately and b is larger value than the a. Staining intensities of m-IDH isozymes showed that the

TABLE 2. *Postulated Genotypes of m-IDH isozyme and Theoretical Ratio of Isozymes Produced in Eeach Genotype.*

Phenotype	Genotype	Ratios of isozymes composed of		
		B1B1B2B2	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

TABLE 3. *Observed Numbers of Phenotypes of m-IDH and the Gene Frequencies of B2 Locus in the Populations of Chum Salmon.*

Population	Number of tested fish	Phenotype (Observed number)			Frequency of *B2* gene	Chi-square
		F	M	S		
Shari	101	60(58.645)	34(36.634)	7(5.721)	0.762	0.5066
Ukedo	100	93(93.123)	7(6.755)	0(0.123)	0.965	0.0213

Number in parentheses represents the expected numbers for each type according to a Hardy-Weinberg equilibrium of $(p+q)^2$.

relative amounts of three bands were in rough accord with their theoretical values.

The distribution of phenotypes of B2 subunit isozyme in the populations of chum salmon in Shari and Ukedo river is shown in Table 3. The frequencies of phenotype were in good agreement with the expectation assuming a Hardy-Weinberg equilibrium of $(p+q)^2$. Thus, it indicates that s-IDH is controlled by two disomic loci. Significant difference of gene frequencies between Shari and Ukedo river populations was demonstrated.

Discussion

A newly arisen tetraploid species has four homologous chromosomes, and the only quadrivalents are found during meiosis. This has been supported by indications for tetrasomic inheritance of several enzyme systems. Such a situation exists in the polymorphism of hexsose-6-phosphate dehydrogenase in brook trout (6), s-NADP-dependent isocitrate dehydrogenase (7), sorbitol dehydrogenase (10), and s-malate dehydrogenase (4) in rainbow trout.

The s-IDH in rainbow trout has been reported to be inherited tetrasomically on the bases of the phenotypic distribution in a population (7). Breeding experiments, however, revealed that in the majority of matings this enzyme was inherited disomically while in others it followed a tetrasomic mode of inheritance (8). Population studies and breeding experiments of the s-IDH in rainbow trout by Allendorf and Utter (11) also indicated a disomic mode of inheritance. Allendorf *et al.* (12) reported that the m-IDH in rainbow trout was represented by three non variant bands indicating the presence of two monomorphic disomic loci with

common alleles of different mobilities.

The present work indicated that s-IDH and m-IDH were controlled by two disomic loci in chum salmon, as well as in rainbow trout. Two different disomic loci for s-IDH were involved in making three molecular forms, while two different disomic loci for m-IDH did not make three molecular forms. The two m-IDH isozymes under the control of two loci showed an identical electrophoretic position.

There is at present no evidence for tetrasomic inheritance but for two disomic inheritance for lactate dehydrogenase, malate dehydrogenase, aspartate aminotransferase, sorbitol dehydrogenase, and phosphoglucomutase in chum salmon (unpublished data). For these reasons, preferential pairing of two definite chromosomes each of the original four homologies seemed to be a probable explanation. The diploidization process in which the ancient tetraploids were involved subsequent to polyploidization, suggests that tetrasomic gene loci become functionally diverse and subject to a disomic mode of inheritance (2). Thus, the behavior of an enzyme IDH confirm the hypothesis that the chum salmon is a tetraploid species in the process of diploidization.

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