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Tohoku Journal of Agricultural Research
Vol. 49 No. 1-2, September 1998
Printed in Japan

Genetic Control of Lactate Dehydrogenese Isozymes of the Japanese Crucian Carp (*Carassius auratus* subsp.)

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(Received, July 7, 1998)

Summary

Genetic control of the lactate dehydrogenase (LDH) isozymes of the Japanese crucian carp (Kinbuna : *Carassius auratus* subsp.) were examined and compared to that of the goldfish (*C. auratus*). The LDH-A, -B, -E, and -F isozymes observed in the electrophoretic pattern of the Japanese crucian carp, were similar to those of the goldfish. However, the LDH-B isozyme in the heart showed weaker activity in the crucian carp than in the goldfish, and the LDH-A isozyme in the heart showed stronger activity in the crucian carp than in the goldfish. Out of these isozymes, only LDH-A and -E showed a fixed single banded phenotype, respectively, but a polymorphism of LDH-B and -F was observed, suggesting that LDH-B isozyme is coded by the two separate loci (*Ldh-B1* and *Ldh-B2*) and LDH-F isozyme is coded by a single locus (*Ldh-F*). The experiments of heat stability and pair mating for these isozymes supported the above suggestion and the genetic difference between the crucian carp and goldfish was revealed.

A previous paper (1) revealed that lactate dehydrogenase (LDH) isozymes of the goldfish (*Carassius auratus*) are controlled at least five loci (*Ldh-A*, *Ldh-B1*, *Ldh-B2*, *Ldh-E*, and *Ldh-F*) throughout the tissue specificity, heat stability, and pair mating experiments. Out of these five loci, *Ldh-A*, *Ldh-E*, and *Ldh-F* were monomorphic. For the remained two loci, *Ldh-B1* and *Ldh-B2*, being duplicate, tow alleles of *A* and *B* were observed at the *Ldh-B1* and a fixed single allele was observed at the *Ldh-B2*.

Takase *et al.* (2) demonstrated the two loci (*6Pgd-1* and *6Pgd-2*) of 6-phosphogluconate dehydrogenase (6PGD) in the Japanese crucian carp (Kinbuna : *C. auratus* subsp.) and in the goldfish to be different in heat stability and not divergent between the two fish. In the Japanese crucian carp, *6Pgd-1* locus was polymorphic exhibiting four different alleles, and *6Pgd-2* locus was monomorphic. On the other hand, in the goldfish, *6Pgd-1* and *6Pgd-2* loci were polymorphic both exhibiting two different alleles in each locus. Takase *et al* (3) also revealed that

three loci (*Gpi-1*, *Gpi-2*, and *Gpi-3*) encoding glucosephosphate isomerase (GPI) in both the Japanese crucian carp and the goldfish throughout the tissue specificity and heat stability experiment, and demonstrated that heat stable allele replacement at *Gpi-1* and *Gpi-2* loci occurs between the two fish.

The purpose of this study is to elucidate the genetic control of LDH isozymes of the Japanese crucian carp by examining the electrophoretic pattern comparing it to the goldfish, to reveal the genetic difference between the crucian carp and goldfish.

Materials and Methods

The 44 specimens of Japanese crucian carp were caught from a natural pond in Miyagi Prefecture. The other 114 specimens were from the laboratory pond of the progeny of the above fish (the 1st and 2nd generations). The 70 specimens of goldfish were purchased from breeders in Yatomi Town in Aichi Prefecture and were kept in our laboratory pond.

Isozyme patttern of lactate dehydrogenase (LDH) were determined by means of a horizontal starch gel electrophoresis. The methods of electrophoresis were based on the discription of Fujio (4). Two buffer systems were adopted as follows: TC-7 (tris-citric acid, pH 7.0) (4) and CAPM (citric acid, 4-3-aminopropylmorpholine, pH 6.0) (5). In all of the fish, the brain, eye, heart, skeletal muscle, and liver were analysed. The nomenclature of isozymes conformed to Markert *et al* (6) and lkeda *et al* (1).

To examine the heat stability in LDH isozymes of the skeletal muscle, the gel after electrophoresis was subjected to heat treatment in a water bath. The procedure of the heat treatment of the gel has been described in a previous paper (2).

For the determination of the genetic control of LDH isozymes, several pairs of the Japanese crucian carp were employed. The offsprings from each pair were kept in a small pond. The offsprings were reared for more than 12 months, until they had reached a size large enough to permit the electrophoretic determination of their isozyme patterns of LDH in the skeletal muscle and liver.

Results and Discussion

Isozyme patterns in skeletal muscle

A survey of the isozyme patterns of skeletal muscle from the 44 natural and 114 cultured specimens of the Japanese crucian carp revealed three different patterns on LDH-A and LDH-B, as shown in Fig. 1. These patterns were similar to the goldfish. These four different patterns designated as type I, II, III, and IV, respectively, on the basis of the description in the previous papar (1). The

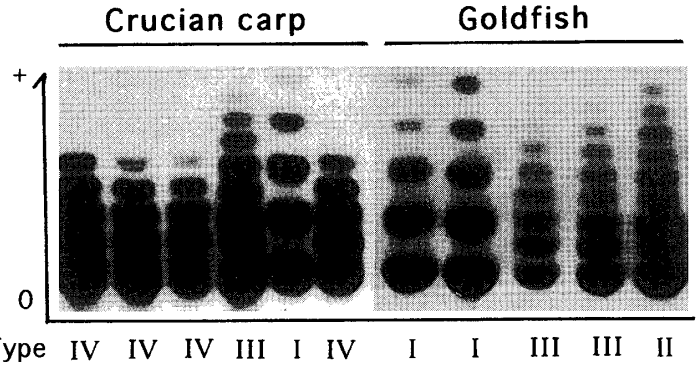

FIG. 1. Four LDH isozyme patterns (I, II, III and IV) of skeletal muscle in the crucian carp and goldfish.

TABLE 1. *Distribution of LDH Isozyme Patterns of the Skeltal Muscle in Crucian Carp and Goldfish*

Isozyme pattern	No. of bands	Crucian carp		Goldfish
		Natural	Cultured	
I	5	0	1	22
II	9	0	0	34
III	9	8	28	14
IV	5	36	85	0
Total		44	114	70

frequencies of these types in both fish are shown in Table 1. Type I and III were observed in both the Japanese crucian carp and the goldfish. Type II was observed in the goldfish but not in the Japanese crucian carp, while type IV was observed predominantly in the Japanese crucian carp but not in the goldfish. The presence of the type IV in the Japanese crucian carp could not be interpreted by the model of the two duplicate loci (*Ldh-B1* and *Ldh-B2*) in the goldfish[1].

Tissue distribution of LDH was examined in the Japanese crucian carp (type IV) and goldfish (type I), as shown in Fig. 2. The five-banded pattern on the anodal side of Japanese crucian carp suggests that the LDH of this fish is a tetrameric molecule and the presence of two separate loci (*Ldh-A* and *Ldh-B*) which code for A- and B-subunits, is the same as the goldfish. However, the activity of these two isozymes in each tissue was different between the two fish. In the Japanese crucian carp, LDH-B was stained weakly but LDH-A was stained strongly in all the tissues. In the goldfish, on the other hand, LDH-A was stained strongly in the brain, eye, skeletal muscle, and the LDH-B was stained strongly in the heart and liver. Especially, in the heart, the LDH-B isozyme showed weaker activity in the Japanese crucian carp than in the goldfish and the LDH-A isozyme showed stronger activity in the Japanese crucian carp than in the

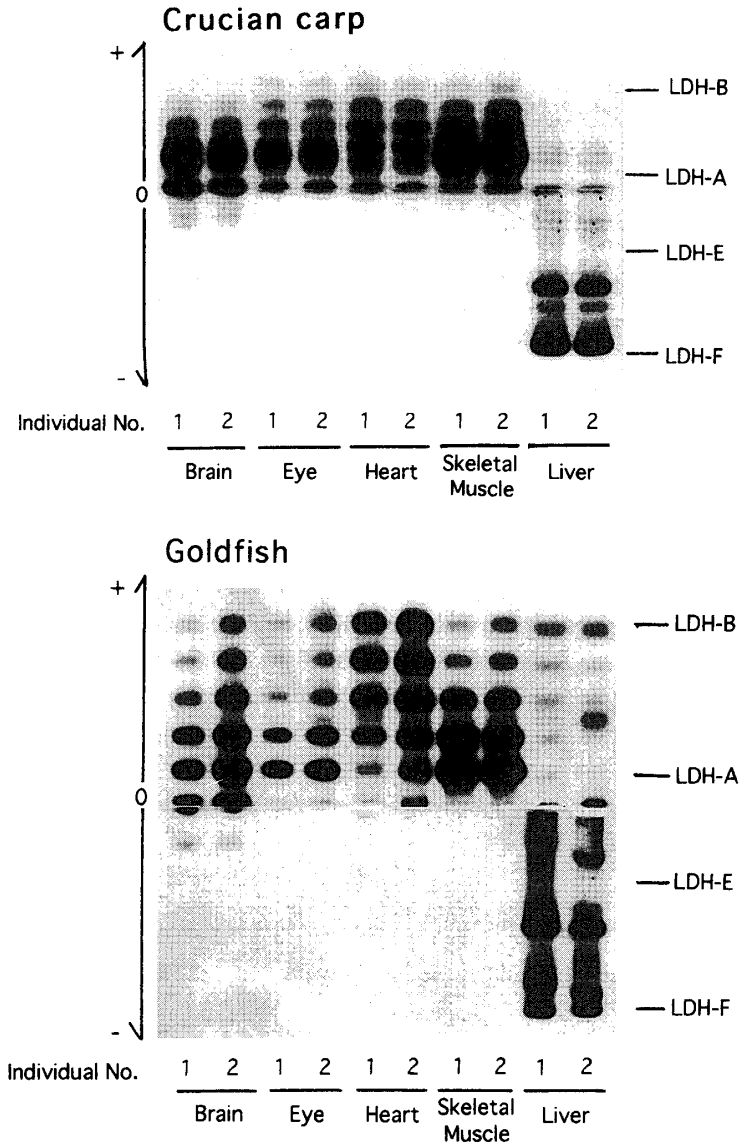

FIG. 2. Tissue specificity of LDH isozyme patterns in the crucian carp and goldfish.

goldfish.

To distinguish clearly between LDH-A and LDH-B, the isozymes of the skeletal muscle were subjected to the heat treatment (Fig. 3). Activity of the LDH-B isozymes was observed at 72°C for 10 minutes. The three tetrameric isozymes composed of A- and B-subunits (hybrid molecules) were not observed in the type I, II, and III of the goldfish. This indicated that the genotypes of type I, II, and III were $B1^AB2^A/B1^AB2^A$, $B1^AB2^A/B1^BB2^A$, and $B1^BB2^A/B1^BB2^A$, respectively. On the other hand, in the Japanese crucian carp, the type IV showed the five banded phenotype after the heat treatment. The most anodal band was different from the B1 subunits coded by *B* allele at the *Ldh-B1* locus of

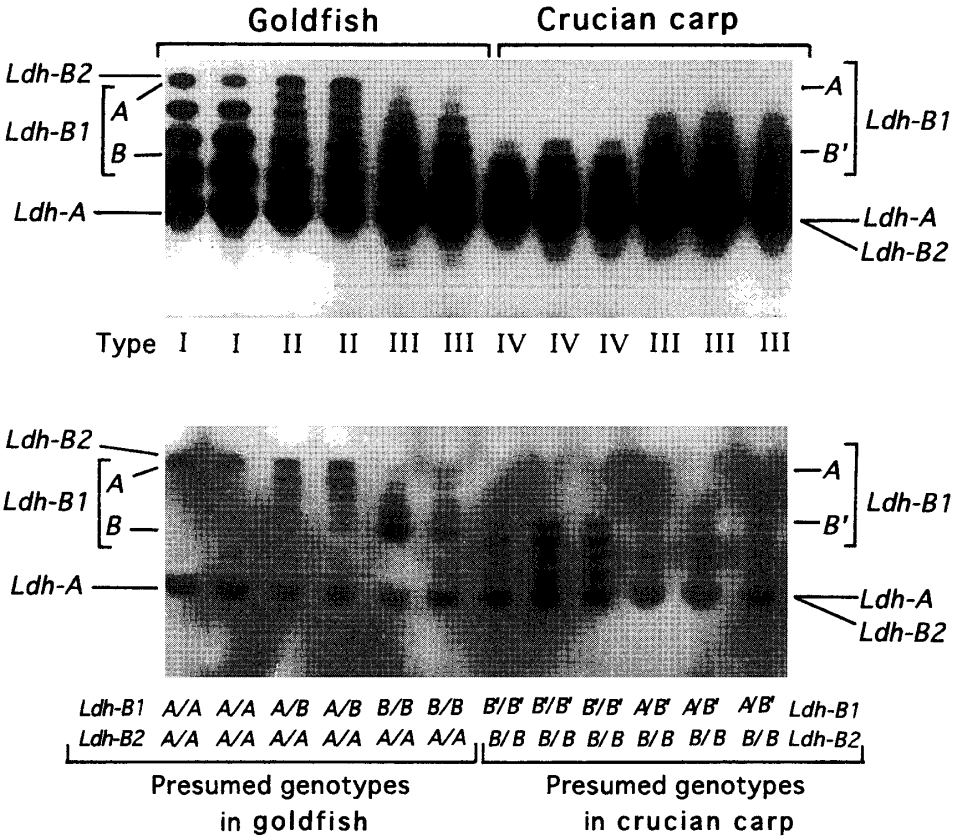

FIG. 3. Heat treatment of LDH isozyme of skeletal muscle in the goldfish and crucian carp. Above pannel: control gel; below pannel: the gel after electrophoresis was heated at 72°C for 10 minutes, and then it was cooled immediately and stained.

the goldfish. This suggests that the most anodal band is a homotetramer of B1 subunits and the least anodal band is both a homotetramer of B2 subunits and a homotetramer of A subunits. If the alleles as *Ldh-B1* are designated as *A* and *B* and the allele at the *Ldh-B2* is designated as *A* in the goldfish, the alleles of the Japanese crucian carp are designated as *A* and *B'* at the *Ldh-B1* locus and as *B* at the *Ldh-B2* locus. The genotypes of type I, III, and IV of the Japanese crucian carp are then interpreted to be $B1^{A}B2^{B}/B1^{A}B2^{B}$, $B1^{A}B2^{B}/B1^{B'}B2^{B}$, and $B1^{B'}B2^{B}/B1^{B'}B2^{B}$, respectively.

The presumed parental genotype combinations at *Ldh-B1* and *Ldh-B2* loci were examined in four pair matings of the Japanese crucian carp with results as shown in Table 2. The genotypes of all offsprings from the pair matings (the cross of $B1^{B'}B2^{B}/B1^{B'}B2^{B}\times B1^{B'}B2^{B}/B1^{B'}B2^{B}$ and $B1^{A}B2^{B}/B1^{B'}B2^{B}\times B1^{B'}B2^{B}/B1^{B'}B2^{B}$) segregated in the expected ratio. All the bands of type III and IV of the offsprings remained as 72°C for 10 minutes.

The results of heat stability and pair mating revealed that the separate *Ldh-B1* and *Ldh-B2* loci estimated in the goldfish exists also in the Japanese

TABLE 2. *Presumed Genotypes of the Parents and Phenotypic Segregation in Their Offspring at Two Ldh Loci (Ldh-B1 and Ldh-B2) in the Crucian Carp*

Cross	Presumed genotypes	Phenotypes of offspring				Expected ratio	χ^2
		I	II	III	IV		
IV×IV	$B1^{B'}B2^{B}/B1^{B'}B2^{B}\times B1^{B'}B2^{B}/B1^{B'}B2^{B}$	0	0	0	115	—	—
IV×IV	$B1^{B'}B2^{B}/B1^{B'}B2^{B}\times B1^{B'}B2^{B}/B1^{B'}B2^{B}$	0	0	0	135	—	—
III×IV	$B1^{A'}B2^{B}/B1^{B'}B2^{B}\times B1^{B'}B2^{B}/B1^{B'}B2^{B}$	0	0	68	52	1 : 1	2.113
III×IV	$B1^{A}B2^{B}/B1^{B'}B2^{B}\times B1^{B'}B2^{B}/B1^{B'}B2^{B}$	0	0	67	53	1 : 1	1.633

crucian carp, and that the mobility of LDH-B2 subunit coded by $Ldh\text{-}B2^{B}$ allele in the crucian carp is the same as the LDH-A. These suggestions help to explain the results of tissue specificity in which the LDH-B isozyme in the heart showed weaker activity in the crucian carp than in the goldfish and the LDH-A isozyme showed stronger activity in the Japanese crucian carp than in the goldfish.

Variants of LDH-F isozyme

From the examination of tissue specificity (see Fig. 2), existence of the LDH-E (brain specific) and LDH-F (liver specific) isozymes revealed in the Japanese crucian carp were the same as the goldfish. In the LDH-E isozyme, variant was not observed in either fish. However, the variants, two single-banded phenotypes and a five-banded phenotype, were observed in the LDH-F isozyme of the Japanese crucian carp (Fig. 4). The two single banded phenotypes were interpreted as the genotypes of A/A and B/B homozygotes, respectively, at the locus of *Ldh-F*. The genotype of the five banded phenotype was thought to be the heterozygote (A/B). On the other hand, in the goldfish, all individuals showed only the genotype of B/B.

The presumed parental genotype combinations at *Ldh-F* locus were examined in three pair matings of the Japanese crucian carp as shown in Table 3. The genotypes of all offspring from the three pair matings (the cross of $A/A\times A/A$, $A/A\times A/B$, and $A/A\times B/B$) segregated in the expected ratio.

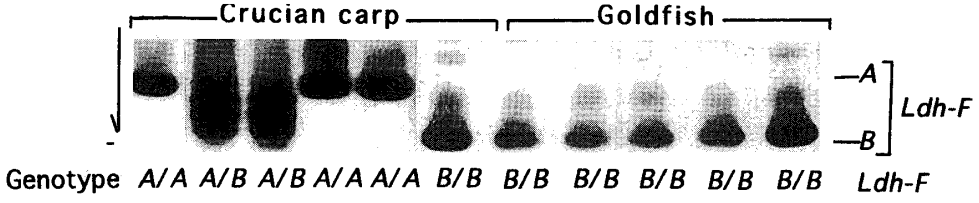

FIG. 4. Variations in LDH-F isozyme in the crucian carp and goldfish.

TABLE 3. *Genotypes of Parents and Their Offsprings at Ldh-F Locus of Crucian Carp*

Cross	Genotypes of offsprings		Total	Expected ratio	χ^2
	A/A	*A/B*			
A/A×*B/B*	0	92	92	—	—
A/A×*A/B*	28	32	60	1 : 1	0.267
A/A×*A/A*	74	0	74	—	—

Genetic differences between Japanese crucian carp and goldfish

The above results revealed that the LDH isozymes of the Japanese crucian carp are controlled by five *Ldh* loci (*Ldh-A*, *-B1*, *B2*, *-E*, and *-F*), similar to the goldfish. Out of these loci, variants ware not observed at *Ldh-A* and *Ldh-E* loci in the Japanese crucian carp and goldfish, but the respective bands were observed in the same position in both fish. At the other three loci (*Ldh-B1*, *-B2*, and *-F*), however, variations within and between both fish were observed. The Japanese crucian carp possesed alleles of $Ldh\text{-}B1^A$, $Ldh\text{-}B1^{B'}$, $Ldh\text{-}B2^B$, $Ldh\text{-}F^A$, and $Ldh\text{-}F^B$, while the goldfish had $Ldh\text{-}B1^A$, $Ldh\text{-}B1^B$, $Ldh\text{-}B2^A$, and $Ldh\text{-}F^B$. The allele frequencies at the five loci in the each fish are as shown in Table 4. Differences of allele frequencies between the two fish were indicated at *Ldh-B1*, *Ldh-B2*, and *Ldh-F* loci. At the *Ldh-B1* and *Ldh-F* loci, the most frequent allele was different between the two fish. The remainig *Ldh-B2* locus was completely divergent between the two fish. A previous paper (3) also found a divergence in *Gpi-1* and *Gpi-2* loci between the two fish. The results of the previous paper and present study demonstrated that a remarkable genetic difference exists between

TABLE 4. *Allele Frequencies of Five Ldh Loci in the Crucian Carp and Goldfish*

Locus	Allele	Crucian Carp		Goldfish
		Natural	Cultured	
Ldh-A	*A*	1.000	1.000	1.000
Ldh-B1	*A*	0.091	0.132	0.557
	B'	0.909	0.868	0
	B	0	0	0.443
Ldh-B2	*A*	0	0	1.000
	B	1.000	1.000	0
Ldh-E	*A*	1.000	1.000	1.000
Ldh-F	*A*	0.716	0.763	0
	B	0.284	0.237	1.000

the Japanese crucian carp and the goldfish.

Acknowledgements

This study was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture of Japan (No. 08406014).

References

1) Ikeda, M., Yamamoto, S. and Fujio, Y., Genetic controls of MDH and LDH isozymes in the goldfish. *Tohoku J. Agr. Res.*, **48**: 43-52 (1997).
2) Takase, T., Kijima, A. and Fujio, Y., Genetic polymorphism and isozyme patterns of 6-phosphogluconate dehydrogenase in goldfish and crucian carp. *Tohoku J. Agr. Res.*, **47**: 67-75 (1997).
3) Takase, T., Kijima, A. and Fujio, Y., Detection of heat stability variants in GPI isozymes of goldfish and crucian carp. *Fisheries Sci.*, **64**: 31-34 (1998).
4) Fujio, Y., "*Study on Genetic Characteristics of Fish and Shellfishes in Isozymic Analysis*", Report of the Special Experimental Research, Ministry of Agriculture and Fisheries of Japan, Tokyo, pp. 65 (1984) (in Japanese).
5) Clayton, J.W. and Tretiack, D.N., Aminecitrate buffers for pH control in starch gel electrophoresis. *J. Fish. Res. Bd. Can.*, **29**: 1169-1172 (1972).
6) Markert, C.L., Shaklee, J.B. and Whitt, G.S., Evolution of a gene. *Science*, **189**: 102-114 (1975).