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Selective Advantage of Heterozygotes at the Liver Isocitrate Dehydrogenase Locus in the Plaice

Siyang LIU, Minoru IKEDA and Yoshihisa FUJIO

Laboratory of Fish Genetics and Breeding Science, Faculty of Agriculture, Tohoku University, Sendai 981, Japan

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Summary

A cultured-released population of the plaice, *Palalichthys olivaceus*, was analyzed by starch gel electrophoresis for liver isocitrate dehydrogenase isozyme (IDH-1). The universal occurence of two alleles (*A* and *B*) at the *Idh-1* locus was observed in 23 different lots from nine different hatcheries. An excess of heterozygotes at the *Idh-1* locus was observed in 19 of 23 different lots, suggesting the selective advantage of heterozygotes. On the assumption that the heterozygotes are 0% in mortality, the survival rates of homozygotes were calculated for the 19 lots showed heterozygote excess. The suvival rate of *A*/*A* and *B*/*B* homozygotes was calculated at an average of 69.2 and 75.2%, respectively. The genotypes were associated with marked differences in survival rate. The standard body length of *A*/*B* heterozygotes was larger than that of *B*/*B* homozygotes in 15 of the 19 lots which showed heterozygotes excess. These results suggest a case of balance polymorphism. The maintenance of stable polymorphism could be explained by the differences of survival and growth rates among the genotypes.

Electrophoretic variants provide a stable means for analyzing the effect of homozygosity or heterozygosity at a random small segment of the genome. One approach to analyzing the effect of genotype is to survey the possible association between the genotypes at one locus and the quantitative traits in individuals drawn randomly from a population.

The previous paper (1) revealed that the frequencies of heterozygotes at the liver isocitrate dehydrogenase (*Idh-1*) locus were often higher than expected under the Hardy-Weinberg's equilibrium in the cultured-released population, but not in the natural population of the plaice (*Paralichthys olivaceus*). This phenomenon might be preferable in explaining the difference of fitness between genotypes. In regard to the above phenomenon, Fujio (2) revealed that the genotypes of the *Idh-1* locus were associated with marked differences in early mortality during the cultured periods ; that is, the survival rate of each homozygote was lower than the total survival rate in the cultured lot of the plaice.

The present work is to confirm the effect of genotypes in survival and growth rates at the liver isocitrate dehydrogenase (*Idh-1*) locus in the cultured-released population of the plaice.

Materials and Methods

Twenty-three lots of the plaice from the cultured-released population produced from 1994 to 1996 were provided from nine different hatcheries in Japan. Most lots were composed of the progeny obtained from a single parental population. Some lots were a mixture of progeny obtained from different parental populations. Standard body length and sampling date are shown in Table 1.

The liver isocitrate dehydrogenase isozyme (IDH-1) was detected by horizon-

TABLE 1. *Collection Data of Cultured-Released Population of the Plaice in This Study*

Lot name	Year of production	Sampling date	No. of individuals	Standard body length Mean±SD (cm)
Aomori	1995	1995. 9.11	108	5.7±0.5
Iwate 1	1994	1994. 8. 9	98	3.3±0.3
Iwate 2	1994	1994. 8. 9	76	2.1±0.4
Iwate 3	1994	1994. 8. 9	90	2.5±0.4
Iwate 4	1995	1995.10.17	93	4.6±2.1
Akita	1995	1995. 4. 9	100	17.7±2.2
Miyagi 1	1994	1994. 8. 9	194	6.4±1.0
Miyagi 2	1994	1994. 8. 9	193	6.2±0.9
Miyagi 3	1995	1995.10.17	100	7.3±1.0
Miyagi 4	1995	1995.10.31	100	8.4±1.1
Miyagi 5	1995	1995.10.31	99	8.3±0.9
Miyagi 6	1995	1995.10.31	100	7.0±0.8
Miyagi 7	1996	1996.10.30	100	7.5±0.8
Fukushima 1	1995	1995.11. 2	100	9.2±0.5
Fukushima 2	1995	1996. 3.22	88	20.6±1.8
Fukushima 3	1995	1996. 3.22	99	18.6±2.4
Fukushima 4	1996	1996.10. 7	100	13.4±1.2
Fukushima 5	1996	1996.10. 7	99	8.7±0.9
Fukushima 6	1996	1996.10. 7	100	9.3±1.5
Kyoto	1995	1995.11.13	100	18.3±1.1
Tottori	1995	1995.11.14	100	20.4±1.4
Kumamoto	1995	1995.11.15	33	22.5±1.6
Miyazaki	1995	1995.11.17	100	12.4±1.7

tal starch gel electrophoresis. The procedures of the electrophoresis and staining were based on Fujio (3).

Results

An excess of heterozygotes

The IDH activity in the liver appeared as two single-banded phenotypes in homozygous individuals and a triple-banded phenotype in heterozygous individuals. The different phenotypes at the *Idh-1* locus indicated two alleles (*A* and *B*) as reported previously (1).

Since this study is focused on an excess of heterozygotes, it is convenient to use an index that assumes positive values when there is an excess of heterozygotes and negative values when there is deficiency. We used the value measured by $(h_o\text{-}h_e)/h_e$, where the h_o is the observed heterozygosity and h_e is the expected heterozygosity.

Table 2 gives the values for 23 different lots in the cultured-released population of the plaice. Nineteen of the 23 lots showed an excess of heterozygotes at the *Idh-1* locus and the deviations from expected values were statistically significant in five of the 19 lots. Thus, a tendency to an excess of heterozygotes was observed. The remaining four lots showed an excess of homozygotes. One of them was a mixture of two lots. Therefore, an excess of homozygotes has been explained by a mixture of the progeny from different populations ("Wahlund effect").

Survival rate of each genotype

If the heterozygotes excess is due to a difference in mortality associated with the genotype, the survival rate should be different for the different genotypes. On the assumption that A/B heterozygote is 0% in mortality, the relative survival rates of A/A and B/B homozygotes were calculated on the basis of the segregation corresponding to the expected ratio under the Hardy-Weinberg's equilibrium. The result is shown in Table 3. In A/A homozygotes, the survival rate was in the range of 14.5 to 99.0, the average being 69.2%. In B/B homozygotes, the rate was in the range of 53.5 to 98.4, the average being 75.2%. The averages were not significantly different between them, but the coefficient of variance was higher in A/A than in B/B.

Relationships between the growth rate and genotypes

If the heterozygotes excess is due to a difference in survival rate associated with the genotype, such differences might be influenced by the growth rate. The differences in the mean standard body length among genotypes of *Idh-1* locus was examined (Table 4). The standard body length of A/B heterozygotes was larger

TABLE 2. *Genotype and Allele Fequency at Idh-1 Locus in Cultured-relessed Population of the Plice*

Lot name	No. of individuals	Genotype frequency			Allele frequency		d value
		A/A	A/B	B/B	qA	qB	
Aomori	108	40(42.9)	56(50.3)	12(14.8)	0.630	0.370	+0.114
Iwate 1	98	19(18.9)	49(48.3)	30(30.8)	0.439	0.561	+0.010
Iwate 2	76	21(13.9)	23(37.2)	32(24.9)	0.428	0.572	−0.382*
Iwate 3	90	20(23.2)	51(45.5)	19(22.3)	0.505	0.495	+0.142
Iwate 4	93	20(19.4)	45(46.2)	28(27.4)	0.457	0.543	−0.024
Akita	100	25(28.1)	56(49.8)	19(22.1)	0.530	0.470	+0.124
Miyagi 1	194	12(27.9)	123(91.3)	59(74.8)	0.379	0.621	+0.350*
Miyagi 2	193	2(11.3)	88(71.7)	103(114.0)	0.239	0.761	+0.255*
Miyagi 3	100	21(25.5)	59(50.0)	20(24.5)	0.505	0.495	+0.180
Miyagi 4	100	8(8.7)	43(41.6)	49(49.7)	0.295	0.705	+0.034
Miyagi 5	99	27(29.2)	54(49.6)	18(21.2)	0.540	0.460	+0.087
Miyagi 6	100	9(17.6)	66(48.8)	25(33.6)	0.420	0.580	+0.355*
Miyagi 7	100	11(11.2)	45(44.6)	44(44.2)	0.335	0.665	+0.009
Fukushima 1	100	25(29.2)	58(49.6)	17(21.2)	0.540	0.460	+0.167
Fukushima 2	88	17(21.0)	52(44.0)	19(23.0)	0.489	0.511	+0.182
Fukushima 3	99	27(29.4)	54(59.1)	18(20.5)	0.545	0.455	+0.099
Fukushima 4	100	47(41.6)	35(45.8)	18(12.6)	0.645	0.355	−0.236*
Fukushima 5	99	18(19.1)	51(48.7)	30(31.2)	0.439	0.561	+0.047
Fukushima 6	100	32(33.6)	52(48.8)	16(17.6)	0.580	0.420	−0.066
Kyoto	100	18(25.5)	65(50.0)	17(24.5)	0.505	0.495	+0.300*
Tottori	100	39(36.0)	42(48.0)	19(16.0)	0.600	0.400	−0.125
Kumamoto	33	3(4.7)	19(15.6)	11(12.7)	0.379	0.621	+0.223
Miyazaki	100	9(16.8)	64(48.4)	27(34.8)	0.410	0.590	+0.322*

Expected number of genotype under the Hardy-Weinberg's equiliblium is indicated in parenthesis.
* Significant difference was observed between the observed and expected numbers of genotypes.
$d=(h_o-h_e)/h_e$

than that of *B/B* homozygotes in 15 of the 19 lots showing an excess of heterozygotes.

Discussion

Kijima *et al.* (4) demonstrated that the polymorphism at *Idh-1* locus was universal in Paralichthydae. In the previous paper (1), universal polymorphism at *Idh-1* is observed in all natural and cultured-released populations of the plaice. The results of the present study indicated that an excess of heterozygotes (*A/B*)

TABLE 3. *Relative Survival Rate of A/A and B/B Homozygotes on the Assumption that A/B Heterozygotes at Idh-1 Locus is 0% in Mortality in Cultured-released population of the Plaice*

Lot name	Survival rate in each genotype		
	A/A	*B/B*	*A/B*
Aomori	83.9	73.2	100
Iwate 1	99.0	95.8	100
Iwate 3	76.9	76.0	100
Akita	79.1	76.6	100
Miyagi 1	32.0	58.5	100
Miyagi 2	14.5	73.5	100
Miyagi 3	69.8	69.2	100
Miyagi 4	88.9	95.3	100
Miyagi 5	85.2	78.3	100
Miyagi 6	37.7	54.8	100
Miyagi 7	97.3	98.4	100
Fukushima 1	73.5	68.8	100
Fukushima 2	68.3	69.9	100
Fukushima 3	83.6	80.0	100
Fukushima 5	90.5	92.0	100
Fukushima 6	89.1	85.1	100
Kyoto	54.2	53.3	100
Kumamoto	51.7	70.5	100
Miyazaki	40.5	58.7	100
Average±SD	69.2±24.1	75.2±13.7	
C.V. (%)	34.8	18.2	

at *Idh-1* locus was observable in the cultured-released population of the plaice. This phenomenon could be explained by a higher suvival and growth rate in heterozygotes (*A/B*) than in homozygotes (*A/A* and *B/B*). The maintenance of universal polymorphism and selective advantage of heterozygotes have been interpreted as being due either to overdominant selection at the isozimic locus or to selection at deleterious loci closely linked to the isozymic locus (associative overdominance). The former interpretation should be rejected because an excess of heterozygotes at *Idh-1* locus is not shown in the natural population of the plaice (1). In regard to the latter interpretation, Ohta (5) has reported theoretically that when many deleterious genes are closely linked to an isozymic locus with two neutral alleles, heterozygotes at this locus tend to show a higher fitness than homozygotes. In a large equilibrium population, the linkage disequilibrium between neutral and deleterious loci can not be observed.

TABLE 4. *Standard Body Length in Each Genotype at Idh-1 Locus of the Cultured-released population of the Plaice*

Lot name	No. of individuals	Standard body length (cm) in each genotype				
		A/A		A/B		B/B
Aomori	108	5.7±0.4(40)	=	5.7±0.6(56)	>	5.6±4.1(12)
Iwate 1	98	3.4±0.3(19)	=	3.4±0.3(49)	>	3.1±0.3(30)
Iwate 3	90	2.5±0.4(20)	=	2.5±0.4(51)	>	2.4±0.4(19)
Akita	100	17.1±1.6(25)	<	17.9±2.4(56)	>	17.8±2.5(19)
Miyagi 1	194	7.3±1.2(12)	>	6.4±1.0(123)	>	6.1+1.1(59)
Miyagi 2	193	6.5±1.5(2)	>	6.1±8.3(88)	<	6.3±0.9(103)
Miyagi 3	100	7.3±1.0(21)	<	7.4±1.1(59)	>	7.1±1.0(20)
Miyagi 4	100	8.8±0.6(8)	<	8.5±1.2(43)	<	8.3±1.0(49)
Miyagi 5	99	8.5±0.9(27)	>	8.1±0.9(54)	<	8.3±0.6(18)
Miyagi 6	100	7.3±0.3(9)	>	6.9±0.8(66)	<	7.2±1.0(25)
Miyagi 7	100	7.1±0.5(11)	<	7.6±0.8(45)	>	7.5±0.8(44)
Fukushima 1	100	9.3±0.5(23)	>	9.2±0.6(58)	>	9.1±0.5(17)
Fukushima 2	88	19.5±2.5(17)	<	21.0±1.5(52)	>	20.6±1.2(19)
Fukushima 3	99	18.7±2.8(27)	>	18.6±2.4(54)	>	18.3±1.8(18)
Fukushima 5	99	8.5±0.9(18)	<	8.9±0.9(51)	>	8.7±0.9(30)
Fukushima 6	100	9.1±1.5(32)	<	9.4±1.5(52)	>	9.2±1.3(16)
Kyoto	100	18.0±0.8(18)	<	18.4±1.1(65)	>	18.2±1.2(17)
Kumamoto	33	22.2±0.9(3)	<	22.6±1.6(19)	>	22.5±1.9(11)
Miyazaki	100	13.2±1.8(9)	>	12.2±1.8(64)	<	12.6±1.2(27)

Number of individuals in each genotype indicated in parenthesis.

In the population which is produced from a finite parental population, however, linkage disequilibrium is generated by genetic drift, and associative overdominance is caused. Indeed, an excess of heterozygotes at *Idh-1* locus is not shown in the natural population of the plaice, and the cultured-released population is significantly more diversified than the natural population at the loci showing rare alleles, suggesting the founder and/or bottleneck effect depending on the number of the parents kept in the hatchery (1). Therefore, the selective advantage of heterozygotes at *Idh-1* in the cultured-released population of the plaice could be explain by the visibility of associative overdominance promoted by founder and/or bottleneck effect. The maintenance of stable polymorphism at *Idh-1* locus of the plaice might be understood as the balance polymrohism of chromosomal sets including *Idh-1* and the loci connected to suvival and growth rates.

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