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Linked Loci with a Null Allele for Liver Esterase in Crucian Carp

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Summary

Isozyme patterns and polymorphism of liver esterase (EST) were examined in crucian carp (*Carassius auratus*). Six electrophoretically different bands were observed and they were named A, B, C, D, E, and F. They were divided into two groups, EST-1 (A, B, and C bands) and EST-2 (D, E, and F) by tissue specificity.

In the EST-1 (A, B, and C bands), the pattern can be seen to be under the control of two alleles, *A* and *a*, *B* and *b*, *C* and *c*, at the three loci, *Est-1*, *Est-2*, and *Est-3*, respectively. In both alleles, the small letter stands for the null allele. After analyzing the parental phenotypes and the phenotypic segregation of their offspring, the linkage between their loci, *Est-1*, *Est-2*, and *Est-3* were revealed.

In the EST-2 (D, E, and F bands), the pattern shows to be under the control of *D* and *d* alleles at the *Est-4* locus and *E and F* alleles at the *Est-5* locus. The parental phenotypes and the phenotypic segregation of their offspring revealed the linkage between both loci, *Est-4* and *Est-5*.

Electrophoretic variants of enzymes are useful as genetic markers for population studies, if the data reliably reflects genetic variation. However, complexities arise in interpretation of population structure analysis from the existense of null alleles. The detection of null alleles at single locus is difficult clue to the absence of activity in the homozygote. Therefore, null allele polymorphism is generally assumed to be rare (1). In the salmonid fish originated from autotetraploidy, the existence of null allele polymorphism has been identified by the absence of activity in the homozygote at one of the duplicate loci (2-6). The existence of null allele polymorphism is also of interest and importance in the diploidization process of duplicate gene loci in the salmonid fish which are thought to have been derived from an autotetraploid ancestor (7-9). Since cyprinid fish showed a diploid-tetraploid relationship, the existence of null allele polymorphism would be expected in crucian carp (*Carassius auratus*) population.

In electrophoretic surveys, liver esterase (EST : EC. 3.1.1.-) showed a phenotypic distribution which is very different among the offspring obtained by pair matings.

The present study proves that this enzyme variation is due to three null alleles which controlled by three loci in one linkage and another linkage.

Materials and Methods

Animal specimens

Isozyme patterns of esterase (EST : EC. 3.1.1.-) were analyzed for crucian carp (Kin-buna : *Carassius auratus*) in Cyprinidae. The fish were caught from a natural pond in Miyagi Prefecture and were propogated in our laboratory pond.

Mating experiments

Pair mating experiments were employed on crucian carp. In the liver of these fish, the isozyme patterns of EST were determined for obtaining the offspring of indivisual pairs. The offspring of indivisual pairs were kept in separate small ponds. They were reared for more than 12 months until they had reached a size large enough to permit the electrophoretic determination of isozyme patterns for EST in livers.

Electrophoresis

The isozyme patterns of EST were determined by means of the horizontal starch gel electrophoresis and staining procedures based on Fujio (10). In all fish, the livers were mainly used. Six electrophoretically different bands were named A, B, C, D, E, and F from the most anodal downwards and each locus was numbered from the most anodal to most cathodal. In this study, the variants at the *Est-1* locus were designated with *A* and *a* to designate the null allele and so on at the *Est-2*, *Est-3*, and *Est-4*. The variants at the *Est-5* locus were designated *E* and *F* allele.

Results

Zymogram of liver esterase

The electrophoretic analysis of brain, eye, heart, skeletal muscle, and liver for EST isozymes showed six electrophoretically different bands and they were named A, B, C, D, E, and F. The tissue specific analysis shows that A, B, and C bands are preferentially expressed in liver and D, E, and F bands are expressed in lever and skeletal muscle (Fig. 1). The former is tentatively designated the EST-1 group and the latter as the EST-2 group. In the EST-1 group, each of the A, B, and C bands showed a presence or an absence of enzyme activity, and absences

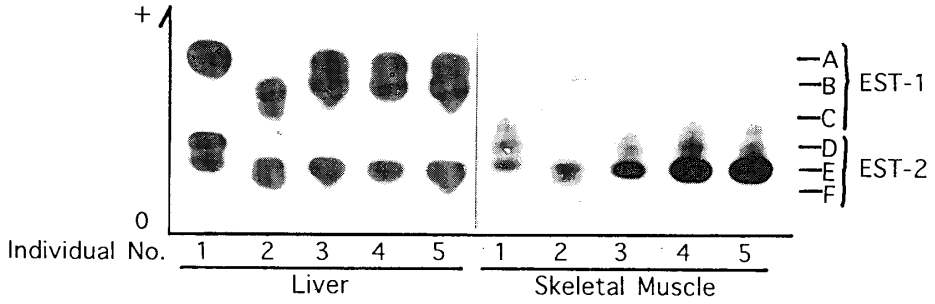

FIG. 1. EST isozyme patterns in liver and skeletal muscle of crucian carp.

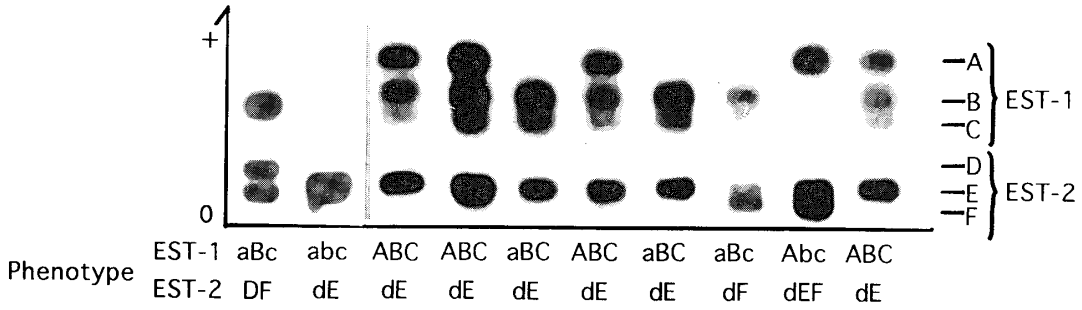

FIG. 2. EST isozyme patterns in liver of crucian carp.

occuring in all three bands over the range of phenotypes were observed (Fig. 2). Six different phenotypes, ABC, ABc, aBC, AbC, Abc, aBc, abC, and abc were determined but AbC and abC phenotypes were not observed (Table 1). In the EST-2 group, each of the D, E, and F bands also showed the presence or absence of enzyme activity as shown in Fig. 2 and six different phentoypes were determined (Table 2). They showed enzyme activity in at least one band and an

TABLE 1. *Phenotypic distribution of EST-1 variants for natural and cultured populations of crucian carp*

Phenotype	Population	
	Natural	Cultured
ABC	4	8
ABc	6	39
aBC	4	11
AbC	0	0
Abc	4	42
aBc	3	12
abC	0	0
abc	1	2
total	22	114

TABLE 2. *Phenotypic distribution of EST-2 variants for natural and cultured populations of crucian carp*

Phenotype	Population	
	Natural	Cultured
DEF	1	13
DE	4	31
DF	2	4
EF	8	23
E	5	43
F	2	0
total	22	114

absence of enzyme activity in both E and F was not observed.

Genetic control of the EST-1 group

From the zymogram mentioned before, three loci are assumed for the three bands in EST-1 group. Assuming three different loci (*Est-1*, *Est-2*, and *Est-3*) for A, B, and c bands, we also assumed the presence of the alleles, *A* and *a*, *B* and *b*, and *C* and *c*, the former being dominant and the latter recessive at each locus.

The following combination of parental genotypes on the basis of *N* (dominant allele) and *n* (recessive allele) will yield the offspring phenotypes in a pair mating system. In case of a parental combinations as follows, $NN \times NN$, $NN \times Nn$ or $NN \times nn$, all offspring will have *N* phenotype which displays the *N* band. And the offspring of $nn \times nn$ mating will have an *n* phenotype which represents the null band. The $Nn \times Nn$ and $Nn \times nn$ crossings will produce the *N* and *n* phenotype distribution in their offspring having expected ratios of 3 : 1 or 1 : 1, respectively. The distribution of the *N* and *n* phenotypes from the offspring in pair mating have a strong correspondence to each of the expected segregation ratios from the presumed parental genotype at each locus (Table 3).

In analyzing the phenotypic distribution of two segregated types AB and ab in mating no. 4 (AB × AB), the segregation ratio of AB and ab was 3 : 1, the ratio being expected for parental genotype combination $AB/ab \times AB/ab$ in the linkage between *Est-1* and *Est-2* loci. In an analysis of the genotypic distribution of two segregated types BC and bc in mating no. 2 (BC × bc), the segregation ratio of BC and bc was 1 : 1, the ratio being expected for parental genotype combination $BC/bc \times bc/bc$ in the linkage between *Est-2* and *Est-3*.

The possible parental genotype combinations, assuming linkage among *Est-1*, *Est-2*, and *Est-3*, were made for each pair mating and these combinations are shown in Table 4.

TABLE 3. *Genotypes of Est-1,Est-2 and Est-3 variants for the offspring in pair matings in crucian carp*

Mating No.	Phenotype Female×Male	Number of examined	*Est-1*				*Est-2*				*Est-3*			
			A-	*aa*	Ratio	χ^2	*B-*	*bb*	Ratio	χ^2	*C-*	*cc*	Ratio	χ^2
1	ABC×abc	76	32	44	(1 : 1)	1.895	44	32	(1 : 1)	1.895	44	32	(1 : 1)	1.895
2	ABC×Abc	27	27	0	(1 : 0)	0	10	17	(1 : 1)	1.815	10	17	(1 : 1)	1.815
3	ABc×Abc	92	92	0	(1 : 0)	0	50	42	(1 : 1)	0.696	0	92	(0 : 1)	0
4	ABc×ABc	57	46	11	(3 : 1)	0.988	46	11	(3 : 1)	0.988	0	57	(0 : 1)	0
5	ABc×aBc	76	39	37	(1 : 1)	0.053	59	17	(3 : 1)	0.281	42	34	(1 : 1)	0.842
6	aBc×aBC	60	0	60	(0 : 1)	0	60	0	(1 : 0)	0	27	33	(1 : 1)	0.600
7	aBc×ABC	39	20	19	(1 : 1)	0.026	32	7	(3 : 1)	1.034	32	7	(3 : 1)	1.034
8	aBc×Abc	42	20	22	(1 : 1)	0.095	18	24	(1 : 1)	0.857	8	34	(1 : 3)	0.794

TABLE 4. *Phenotypic distribution of EST-1 for the offspring in pair matings in crucian carp*

Mating No.	Phenotype Female × Male	ABC	ABc	aBC	Abc	aBc	abc	Total	χ^2	Presumed genotype of the parents
1	ABC × abc	0	0	44 (38.0)	32 (38.0)	0	0	76	1.895	*Abc/aBC* × *abc/abc*
2	ABC × Abc	10 (13.5)	0	0	17 (13.5)	0	0	27	1.815	*aBC/Abc* × *Abc/Abc*
3	ABc × Abc	0	50 (46.0)	0	42 (46.0)	0	0	92	0.696	*ABc/Abc* × *Abc/Abc*
4	ABc × ABc	0	46 (42.75)	0	0	0	11 (14.25)	57	0.988	*ABc/abc* × *ABc/abc*
5	ABc × aBC	22 (19.0)	0	20 (19.0)	17 (19.0)	17 (19.0)	0	76	0.947	*Abc/aBc* × *aBC/abc*
6	aBc × aBC	0	0	27 (30.0)	0	33 (30.0)	0	60	0.600	*aBc/aBc* × *aBC/abc*
7	aBC × ABC	13 (9.75)	0	19 (19.5)	7 (9.75)	0	0	39	1.615	*aBC/abc* × *Abc/aBC*
8	aBc × Abc	0	8 (10.5)	0	12 (10.5)	10 (10.5)	12 (10.5)	42	1.048	*aBc/abc* × *Abc/abc*

TABLE 5. *Genotypes of Est-4 and Est-5 variants for the offspring in pair matings in crucian carp*

Mating No.	Phenotype	Number of examined	*Est-4*				*Est-5*					Presumed genotype of the parents
	Female × Male		*D-*	*dd*	Ratio	χ^2	*EE*	*EF*	*FF*	Ratio	χ^2	
1	DEF × E	76	36	40	(1 : 1)	0.211	40	36	0	(1 : 1 : 0)	0.211	*DE/dF × dE/dE*
8	DE × EF	42	24	18	(1 : 1)	0.857	21	21	0	(1 : 1 : 0)	0	*DE/DE × dE/dF*
2	E × F	27	0	27	(0 ; 1)	0	27	0	0	(1 : 0 : 0)	0	*dE/dE × dE/dE*
4	EF × EF	57	0	57	(0 : 1)	0	15	31	11	(1 : 2 : 1)	1.000	*dE/dF × dE/dF*
7	EF × E	39	0	39	(0 : 1)	0	22	17	0	(1 : 1 : 0)	0.641	*dE/dF × dE/dE*
5	EF × E	76	0	76	(0 : 1)	0	42	34	0	(1 : 1 : 0)	0.842	*dE/dF × dE/dE*
6	E × EF	60	0	60	(0 : 1)	0	32	28	0	(1 : 1 : 0)	0.267	*dE/dE × dE/dF*
3	EF × F	92	0	92	(0 : 1)	0	0	45	47	(0 : 1 : 1)	0.043	*dE/dF × dF/dF*

Genetic control of the EST-2 group

From the zymogram of the EST-*2* group mentioned before, two loci (*Est-4* and *Est-5*) are assumed for D, E, and F bands. The pattern shows to be under the control of two alleles, *D* and *d*, at the *Est-4* locus and two alleles, *E* and *F*, at the *Est-5* locus (Table 5). *E* allele frequency at the *Est-5* locus was 0.614 and 0.807 and *F* allele frequency was 0.386 and 0.193 in natural and cultured populations, respectively. The genotypic frequencies at the *Est-5* indicated that the natural and cultured population were under Hardy-Weinberg equilibrium.

In analyzing the phenotypic distribution of DE, dEF, dE, and DEF in mating no. 1 ($DE/dF \times dE/dE$), the result obtained significantly differed from the expected ratio of 1 : 1 : 1 : 1 for an independent segregation of the genes at *Est-4* and *Est-5* and indicated the linkage between *Est-4* and *Est-5* (Table 6). The recombinants between dE and DEF phenotypes are expected but DEF can not be counted. The recombination frequency between *Est-4* and *Est-5* loci was estimated using the recombination data of segregation in mating no. 1 and it turned out to be 3.9%.

Discussion

The present inheritance of the *Est-1*, *Est-2*, *Est-3*, and *Est-4* genes is given by two alleles for each loci, *N* and *n*, standing *N* for dominant and *n* for recessive or null allele. Such an inheritance has been reported in the multiple banded phenotypes of leucine aminopeptidase in apple snail (11). The existence of null alleles in considered to be rare (1) and difficult to detect (12). They have mainly been detected in salmonid species (2-6). Fujio and Macaranas (13) found a null allele for malate dehydrogenase in guppies, describing that this homozygote can exist in one of the duplicated loci, because the other locus is able to synthesize the isozyme resulting in normally functioning proteins.

The finding of linked null alleles, *a*, *b*, and *c* at *Est-1*, *Est-2*, and *Est-3* in crucian carp reveals many interesting issues on genetic research for this fish. One of them will be to find out if this phenomenon is due to be complemental by the

TABLE 6. *Segregation of phenotypes in EST-2 group in mating no. 1 ($DE/dF \times dE/dE$)*

Phenotype (Genotype)	Offspring	
DE (*DE/dE*)	36	Non-recombinant
EF (*dE/dF*)	37	
E (*dE/dE*)	3	Recombinant
DEF (*DF/dE*)	0	
Total	76	

Est-5 locus, the underlying mechanism that produces the homozygote for linked three null alleles at the three loci, *Est-1*, *Est-2*, and *Est-3*, in this fish.

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