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Allozyme Markers for Population Structure Analysis in *Labeo dero* (Hamilton Buchanan, 1822)

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Abstract

Labeo dero specimens, from four different rivers (n= 60), were analysed for allozyme variations. The eighteen enzyme systems screened provided twenty five scorable loci. Seven loci were polymorphic. The significant genotype heterogeneity was observed, between the sample sets. The identified loci exhibited significant potential, to determine population structure of *L. dero*, across its natural range of distribution.

Introduction

Labeo dero (Cyprinidae) is an important food fish and inhabits fresh water streams at the foothill regions. It is distributed in the rivers originating from the Himalayan ranges, across China, Myanmnar, India, Nepal and Pakistan. In India, it is distributed in the river systems of the Indus

and Ganga and is categorized among the vulnerable species of India (Mahanta et. al. 1994). At present, the contribution of *L. dero* to fisheries is through capture from natural resources. However, it is identified as a potential cultivable species. In view of the significance attached to the species, planned conservation of natural populations and fisheries management is necessary. To plan the appropriate strategy, necessary documentation of polymorphic markers and pattern of genetic variation across its natural distribution range, needs to be explored. Available literature on biology and other studies on *L. dero* have been reviewed by Chonder (1999). The genetic research on the species is limited, restricted to karyotyping only (Khuda-Baksh and Chanda 1989). There is no recorded information on genetic markers and distribution of genetic variation in the natural population of *L. dero*.

Allozyme loci are proven tools to determine population structure and estimate intrapopulation gene flow in natural fish populations. These have been successfully used to determine stock structure of several fish species (Menezes 1993, Jerry 1997, van der Bank et. al. 1997, Cashihlo and McAndrew 1998, Hawkins et. al. 2002).

The present study analyses allozyme markers, to identify the polymorphic loci in *L. dero*. Significance of the identified loci to determine genetic divergence in the natural populations of *L. dero* is also assessed.

Materials and Methods

L. dero specimens were collected through commercial catches from four rivers, Beas (Pathankot, n=15), Ganga (Luksar, n=15), Yamuna (Yamunanagar, n=15) and Jiabharali - Tributary of Brahmaputra (Bhaluk Pong, n=15). River Beas is a part of the westward flowing Indus river system and other three are distant but associated rivers of eastward flowing Ganges (ECAFE 1966). The specimens were dissected at site to excise tissue (liver and muscle) samples. The samples were immediately immersed in liquid nitrogen (-196ºC). The tissue samples in frozen state were transported to the laboratory and stored at –80ºC, until used for allozyme analysis.

Enzyme systems were assessed using vertical polyacrylamide gel electrophoresis (Amersham Biosciences Ltd.). Gels of 10x8 cms size were used for all the enzymes except esterases, that were resolved on 10x12 cms size gel.

L. dero was being explored for allozyme variations first time. Therefore, optimization of electrophoretic conditions was necessary to score allelic variation, with good resolution and band intensity. The different parameters standardized for the study are given in table 1.

For the enzyme systems studied, muscle samples did not provide any additional loci or better resolution of any locus, in comparison to that observed in liver. Therefore, the liver was chosen as the optimum tissue. Other conditions that were found optimum, are given in table 1. Extract application volume (ml) and electrophoresis running time (min.) were optimized for individual enzyme, after other parameters have been selected.

The optimized parameters were used to resolve electrophoretic patterns of different enzymes. The liver tissue (60-80 mg) was crushed mildly and homogenized with extraction medium @ 500 $mg \cdot ml^{-1}$. Homogenates were centrifuged (SIGMA Laborzentrifugen GmbH, Osterode) for 1 hour at 10,000 rpm at 4ºC and the supernatant was subjected to recentrifugation for 20 min. Supernatant extract (1-3 ml) was loaded in the gels. Electrophoresis runs were carried out at 150V in cooled chamber (4ºC) using TBE (D) running buffer. Extract volume and electrophoresis running time for individual enzyme is given in table 2. Allozyme profiles were visualized using histochemical staining methods (Whitmore 1990).

Nomenclature of loci and alleles was followed as recommended by Shaklee et al. (1990). At all the loci, most common allele was assigned as 100. Alternate alleles were designated as per their mobility, relative to the most common allele. Parameters of genetic variation like proportion of polymorphic loci ($P_{0.95}$ and $P_{0.99}$) in each population, heterozygosity at individual locus and mean overall loci for each population were calculated with GENETIX (version 4.0, Belkhir et al. 1997). GENEPOP version 3.3d (Raymond and Russet 1995) was used to assess conformity of allele frequencies to that expected under Hardy-Weinberg equilibrium and genic differentiation.

Table 1. Parameters optimized for allozyme electrophoresis in *Labeo dero*

Sl. No.	Parameters	Variation tested	Found optimum
1	Tissue	Liver/Muscle	Liver
2	Tissue(mg)/extraction medium[*1] (ml)	250 $mg \cdot ml^{-1}$, 333 $mg \cdot ml^{-1}$ and 500 $mg \cdot ml^{-1}$	500 $mg \cdot ml^{-1}$
3	PAGE concentraction	7, 8, 9 %	8 %
4	Running buffer	TBE (D)[*2], TCE (D)[*3]	TBE (D)
5	Extract volume loaded	1, 3, 6 ml	Individual enzyme table 2
6	Running time	45-150 min.	Individual enzyme Table 2

*[1]-Extraction medium - Liver- (0.17 M Sucrose, 0.2 M EDTA, 0.2 M Tris-HCl, pH 7.0) and Muscle- 10 % Sucrose

*[2]- 1 X TBE(D) - 50mM Tris, 65 mM Boric acid, 1.6 mM EDTA pH 8.0

*[3]- 1X TCE(D) - 50mM Tris, 65 mM Citrate, 1.6 mM EDTA pH 8.0

Results and Discussion

The 18 enzyme systems studied, provided 25 scorable loci (Table 2). Seven loci, *EST*-1*, *EST*-2*, *G6PD**, *GPDH**, *PGDH**, *XDH**, and *GPI** were found polymorphic, over all the population samples studied. A locus was considered polymorphic, if the frequency of most common allele was £ 0.99 (Hartl 1988). The remaining loci were monomorphic in all the populations studied. The heterozygote genotype for the polymorphic loci confirmed to the banding pattern, expected as per the known structure of the respective enzymes (Whitmore 1990). Loci *EST*-1, EST* -2, G6PD*, GPI** and *GPDH** exhibited three allele variants whereas the other two loci, *PGDH**and *XDH**, were represented by two alleles.

Parameters of genetic variation at each enzyme locus in *L. dero* are given in table 3. The mean number of alleles per locus ranged from 1.28 (Yamuna) to 1.40 (Ganga). The percentage of polymorphic loci differed between different populations and ranged from 0.217 (Beas) - 0.280 (Ganga). The observed heterozygosity per locus among the four riverine populations varied between 0.049 (Brahmputra) and 0.077 (Yamuna). These values were found to be consistent with value range described for teleostean fishes (Nevo 1978). Locus *XDH** (Beas) did not conform to Hardy-Weinberg equilibrium (P= 0.0021, <0.0029) after the critical probability levels were adjusted for sequential bonferroni adjustment. The

Table 2. Enzyme E.C. numbers and scorable loci with sample extract volume and running time of electrophoresis found optimum in *Labeo dero*.

Enzyme Loci	E.C number (µl)	Loci volume (min)	Sample time	Running
α-Glycerophosphate dehydrogenase	1.1.1.8	*GPDH**	1	105
Acid phosphatase	3.1.3.2	*ACP**	3	80
Adenylate kinase	2.7.4.3	*AK**	3	100
Aspartate amino transferase	2.6.1.1	*AAT **	3	70
Creatine kinase	2.7.3.2	*CK**-1,-2	3	120
Esterase	3.1.1.1	*EST **-1,-2	1	130
Glucose dehydrogenase	1.1.1.47	*GLDH**-1,-2	3	100
Glucose phosphate isomerase	5.3.1.9	*GPI**	1	105
Glucose-6-phosphate dehydrogenase	1.1.1.49	*G6PDH**	1	130
Glutamate dehydrogenase	1.4.1.3	*GDH**	3	105
Lactate dehydrogenase	1.1.1.27	*LDH**	3	145
Malate dehydrogenase	1.1.1.37	*MDH**-1,-2	1	105
Malic enzyme	1.1.1.4	*ME**-1,-2	1	105
Octonol dehydrogenase	1.1.1.73	*ODH**	3	105
Phosphogluco mutase	5.4.2.2	*PGM**-1,-2	3	105
Phosphogluconate dehydrogenase	1.1.1.44	*PGDH**	1	105
Superoxide dismutase	1.15.1.1	*SOD**-1,-2,-3	3	100
Xanthine dehydrogenase	1.1.1.204	*XDH**	1	145

present data attributed this to the deficiency of heterozygotes indicated by +ve F_{is} value (fixation index).

Test for genic differentiation over all samples was performed to test the null hypothesis that the samples have homogenous allele frequencies. Significant heterogeneity was observed ($P < 0.05$) at four loci (***EST*-1***, ***G6PD****, ***XDH**** and ***PGDH****). Combined probability over all loci was highly significant ($P < 0.0001$) indicating that the four sample sets might not belong to a single homogenous random mating population (Table 3). Nevertheless, larger sample size from more locations may be required to conclusively document the stock structure of *L. dero* and genetic bottlenecks, if any occurring in the natural populations.

Table 3. Parameters of genetic variation for four riverine populations at each allozyme locus in *Labeo dero*.

Locus	Population	Alleles	Hexp.	Hobs.	HW (p)	Genic heterogeneity (p)
GPDH*	B	1 (100)	0.000	0.000	-	0.7060
	Y	1 (100)	0.000	0.000	-	
	J	2 (84,100)	0.064	0.067	-	
	G	2 (100.113)	0.074	0.077	-	
EST* -1	B	2 (100,106)	0.124	0.000	0.035*	0.0001**
	Y	2 (100,106)	0.124	0.133	1.000	
	J	3 (92,100,106)	0.504	0.200	0.006*	
	G	3 (92,100,106)	0.184	0.067	0.035*	
EST* -2	B	3 (100,108,113)	0.491	0.400	0.159	0.1607
	Y	3 (100,108,113)	0.429	0.071	0.077	
	J	3 (100,108,113)	0.433	0.200	0.139	
	G	3 (100,108,113)	0.300	0.333	0.034*	
GPI*	B	1 (100)	0.000	0.000		1.0000
	Y	2 (85,100)	0.064	0.067		
	J	1 (100)	0.000	0.000		
	G	2 (100,115)	0.064	0.067		
G6PDH*	B	3 (97,100,112)	0.460	0.467	0.183	0.0001**
	Y	2 (100112)	0.480	0.533	1.000	
	J	2 (100112)	0.358	0.200	0.123	
	G	3 (97,100,112)	0.551	0.267	0.007*	
PGDH*	B	2 (100,113)	0.231	0.267	1.000	<0.0001**
	Y	2 (100,113)	0.490	0.429	0.621	
	J	1 (100)	0.000	0.000	-	
	G	2 (100,113)	0.391	0.400	1.000	
XDH*	B	2 (100,108)	0.420	0.067	0.002**	0.0288*
	Y	2 (100,108)	0.491	0.333	0.294	
	J	2 (100,108)	0.444	0.267	0.232	
	G	2 (100,108)	0.359	0.333	1.000	
Mean over all loci	B	1.304	0.075	0.052	-	<0.0001
	Y	1.280	0.088	0.077	-	
	J	1.304	0.087	0.049	-	
	G	1.400	0.092	0.062	-	

Significant at ($P<0.05$), ** significant after critical significance level is adjusted for sequential bonferroni correction (Lessios 1992)

B - Beas; Y - Yamuna; J - Jiabharali; G - Ganga.

Seven polymorphic loci were identified through the present allozyme analysis in *L. dero*. The identified loci exhibited significant promise to document fine scale population structure of *L. dero,* across its natural range of distribution.

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