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Note

Identification of allozyme markers for population structure analysis in *Cirrhinus mrigala* (Hamilton-Buchanan, 1882).

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ABSTRACT

Seven polymorphic allozyme loci were identified in *Cirrhinus mrigala*. The genetic variation detected at each allozyme locus was assessed for samples collected from five rivers. The observed heterozygosities per locus ranged from 0.100 to 0.146. Significant genotype heterogeneity indicated that the samples are not drawn from same genepool. The results suggest the potential of the identified loci to analyse stock structure of natural population of *C. mrigala*.

Cirrhinus mrigala inhabits freshwater rivers and water bodies, distributed naturally in the rivers of the Indus and Ganga system. It is an important food fish and extensively cultivated with other Indian major carps. *C. mrigala* aquaculture contributes to 5,73,294 mt, approximately 1.61% of total world fish production (FAO, 2000). Chonder (1999) reviewed the biology and other information about the fish. Genetic studies on the species are limited to the species specific differences using various techniques such as, Karyotyping (Lakra and Krishana, 1996), DNA fingerprinting through Bkm and M13 probes (Majumdar *et al*., 1997), RAPD (Zheng *et al*., 1999), MboI satellite (Padhi *et al*., 1998) and esterases (Gopalakrishanan *et al*., 1997). However, no information is available on polymorphic protein or DNA markers and genetic variation in *C. mrigala* population across the range of natural distribution. The information is vital for planning, management and conservation of natural resources, besides it is useful in genetic improvement programmes. The present study analyses allozyme markers in *C. mrigala*, to identify the polymorphic loci. Suitability of identified polymorphic allozyme loci in analyzing population structure of *C. mrigala* was also assessed.

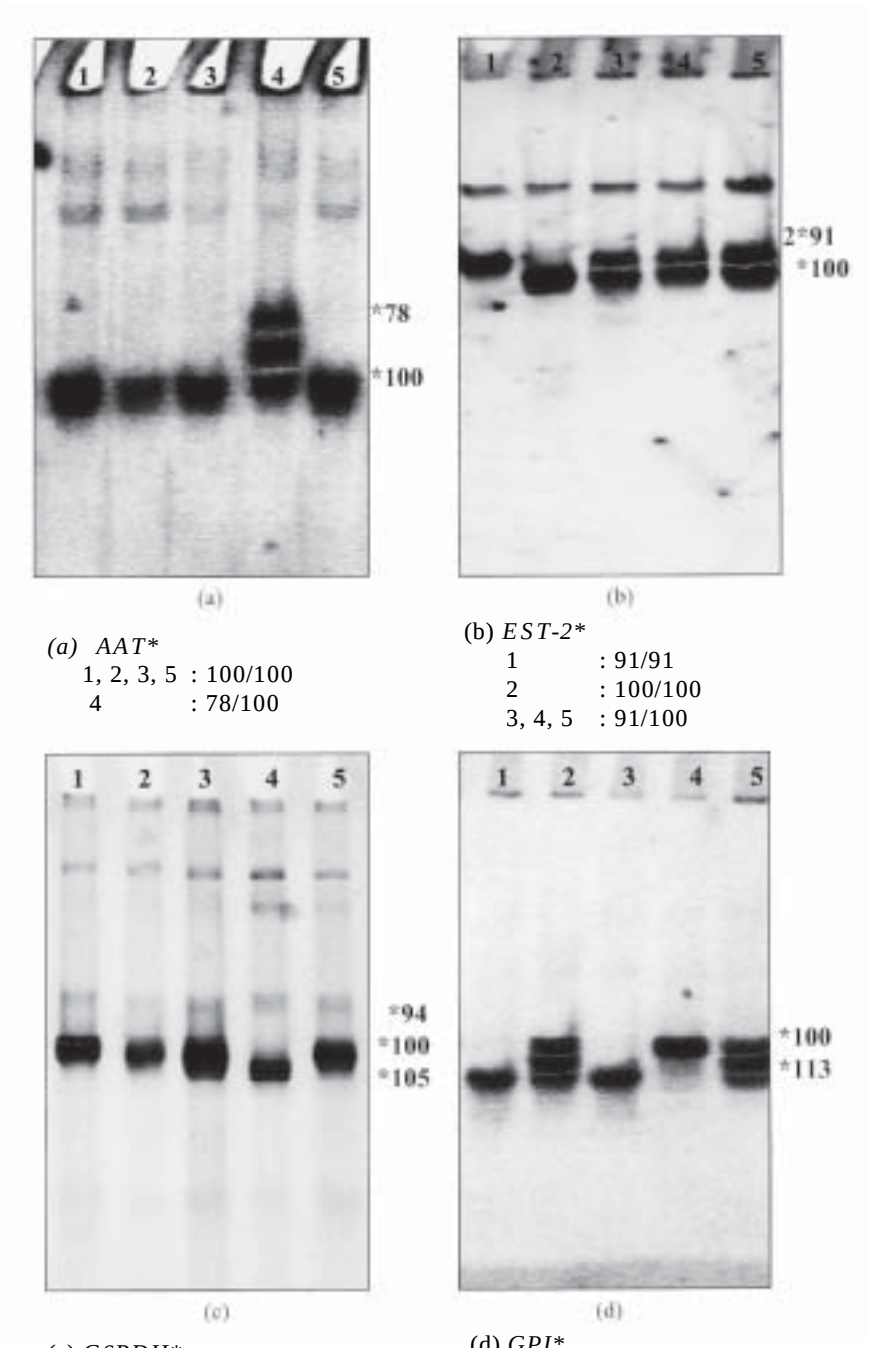
Specimens of *C. mrigala* were obtained through commercial catches from five rivers *viz*., Satluj, Ghagra, Gomti, Bhagirathi and Brahmaputra. Satluj is part of Indus river system and other four rivers are distant but associated rivers of the Ganges (ECAFE, 1966). Sampling sites provide wide coverage across the natural distribution range of *C. mrigala*. The liver tissue were collected from five riverine populations of *C. mrigala* and immediately frozen in

liquid nitrogen (- 196° C). The samples were transported to lab and stored at -80° C till analysis. Frozen liver samples (approximately 100 mg) were homogenized in 250 mg ml^{-1} extraction buffer (0.17M Sucrose, 0.2M EDTA, 0.2M Tris-HCl, pH 7.0). Homogenized samples were centrifuged for an hour at 10,000 rpm at 4° C and the supernatant was recentrifuged for 20 minutes. Allelic variation was investigated using 7% polyacrylamide gel electrophoresis. Electrophoresis was carried out at constant voltage of 150V at 4° C. A total of 25 enzyme systems were examined and 16 enzymes were found to give scorable activity. Visualization of different alleles of enzymes was done by histochemical staining (Whitmore, 1990). Loci and alleles were designated (Shaklee *et al.*, 1990). To investigate the genetic variability, 102 fish samples (24-25 each from five rivers) were genotyped for each allozyme locus. The genotype data was analysed with software Genepop, to estimate parameters of genetic variation and heterogeneity (Raymond and Rousset, 1995a). The allele frequencies, heterozygosities (H_{obs} and H_{exp}) were calculated. The genotype heterogeneity over all loci and conformity to Hardy-Weinberg expectations were also estimated.

Total sixteen enzyme systems were examined that yielded 23 scorable loci. Sixteeen of twenty three loci were monomorphic represented by a single allele in all the populations. Seven loci (30.4%), *AAT**, *EST-2**, *GPDH**, *G6PDH**, *GPI**, *ODH**, *XDH** were polymorphic (Table 1). The banding pattern of heterozygotes in polymorphic loci confirmed to that expected as per the structure of the respective protein (Whitmore, 1990). Loci *G6PDH** and *GPI**, were expressed by three alleles, whereas *AAT**, *EST-2**, *GPDH**, *ODH** and *XDH** were represented by two alleles each (plate 1 and 2). Five loci *EST-2**, *GPDH**, *G6PDH**, *GPI** and *XDH** were polymorphic in all the populations. Locus *ODH** was polymorphic in four populations (except in Gomti samples), while *AAT** exhibited alternate allele only in the samples of River Gomti. Parameters of genetic variation are given in table 2. The percentage of polymorphic loci using the $P_{0.99}$ criteria ranged from 22.7-31.8% and the mean number of alleles per locus ranged from 1.27 to 1.36. The observed heterozygosity per locus varied from 0.100 (in Bhagirathi samples) to 0.146 (in Brahmaputra samples). The observed allele frequencies did not deviate significantly from the Hardy Weinberg proportion indicating that assumption of random mating etc may not be violated. Genetic homogeneity of five sample sets was determined through an exact test (G based test) that assumes random samples of genotypes. This test is performed on genotype tables and possible non- independence of alleles within genotypes will not affect test validity (Raymond and Rousset, 1995b; Goudet *et al.*, 1996).Combined probability over all loci and sample sets was found to be 0.017, indicating that the sample sets differ significantly ($P<0.05$) in their allele frequencies. This indicates that the different sample sets are not drawn from single panmictic population and distinct population sub structuring in *C. mrigala* may be possible across its range of distribution. The estimates provided strong evidence that the genetic variation detected at the identified allozyme loci may be useful in stock structure analysis of *C. mrigala*. The results of the study clearly identified seven polymorphic loci. The identified loci exhibit promise for use in stock structure analysis of *C. mrigala* across its range of distribution.

TABLE 1: *Enzyme loci, enzyme commission (E. C.) number and observed alleles for allozyme analysis in* Cirrhinus mrigala. *The enzymes marked 'ns' did not yield any scorable activity.*

Loci	**E. C. number**	**Locus**	**Alleles**
Acid phosphatase	3.1.3.2	*ACP**	ns
Adenylate kinase	2.7.4.3	*AK**	100
Alcohol dehydrogenase	1.1.1.1	*ADH**	ns
Aldolase	4.1.2.13	*ALDO**	ns
Alkaline phosphate	3.1.3.1	*ALP**	ns
Aspartate amino transferase	2.6.1.1	*AAT**	078, 100
Creatine kinase	2.7.3.2	*CK**	ns
Esterase	3.1.1.1	*EST-1**	100
		*EST-2**	091, 100
Fumerase	4.2.1.2	*FUM**	ns
Glutamate dehydrogenase	1.4.1.3	*GDH**	100
Glucose-6-phosphate dehydrogenase	1.1.1.49	*G6PDH**	094, 100,108
Glucose phosphate isomerase	5.3.1.9	*GPI**	100, 105, 103
Glucose dehydrogenase	1.1.1.47	*GLDH**	100
a-Glycerophosphate dehydrogenase	1.1.1.8	*GPDH**	082, 100
Glyceraldehyde-3-Phosphate	1.2.1.12	*GAPD**	ns
Dehydrogenase			
Hexokinase	2.7.1.1	*HK**	ns
Isocitrate dehydrogenase	1.1.1.42	*ICD**	100
Lactate dehydrogenase	1.1.1.27	*LDH-1**	100
		*LDH-2**	100
		*LDH-3**	100
Malic enzyme	1.1.1.40	*ME-1**	100
		*ME-2**	100
Octonol dehydrogenase	1.1.1.73	*ODH**	048, 100
Phosphogluconate dehydrogenase	1.1.1.44	*6PGD**	100
Phosphogluco mutase	5.4.2.2	*PGM-1**,	100
		*PGM-2**	100
Pyruvate kinase	2.7.1.40	*PK**	ns
Superoxide dismutase	1.15.1.1	*SOD-1**	100
		*SOD-2**	100
	*SOD-3**	100	
Xanthine dehydrogenase	1.1.1.204	*XDH**	93, 100



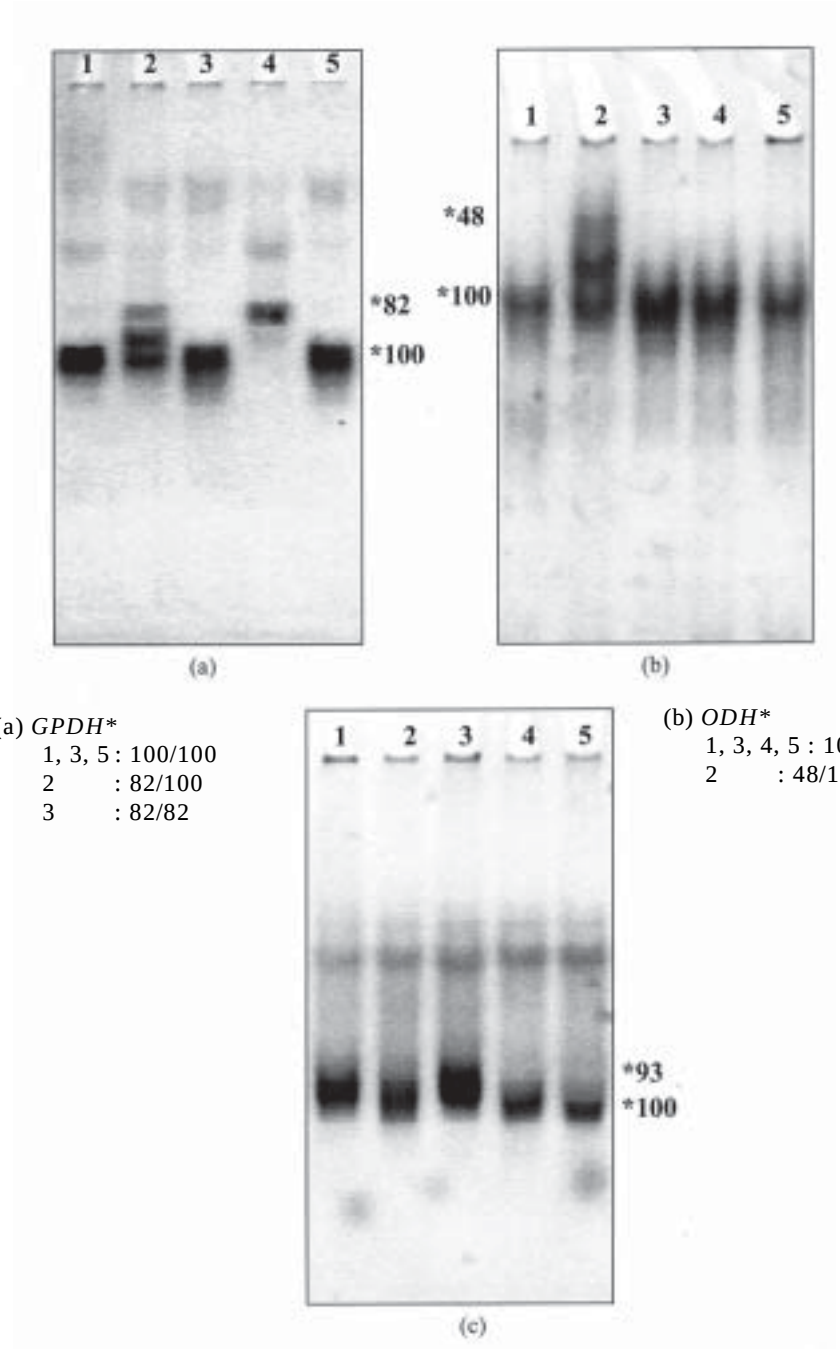
*(a) AAT**
1, 2, 3, 5 : 100/100
4 : 78/100

(b) *EST-2**
1 : 91/91
2 : 100/100
3, 4, 5 : 91/100

(c) *G6PDH**
1, 3, 5 : 94/100
2 : 100/100
4 : 100/105

(d) *GPI**
1, 3 : 113/113
2, 5 : 100/113
4 : 100/100

Plate 1: Polymorphic allozyme loci and different genotypes observed in *C.mrigala*. 1-5 different individuals.



(a) *GPDH**
1, 3, 5 : 100/100
2 : 82/100
3 : 82/82

(b) *ODH**
1, 3, 4, 5 : 100/100
2 : 48/100

(c) *XDH**
1 : 93/93
2, 4, 5 : 100/100
3 : 93/100

Plate 2: Polymorphic allozyme loci and different genotypes observed in *C. mrigala*. 1-5 different individuals.

TABLE 2: *Parameters of genetic variation in* Cirrhinus mrigala.

Riverine populations	Mean heterozygosity values		% of polymorphic loci		Mean number of alleles per locus
	H_{exp}	H_{obs}	P(0.95)	P(0.99)	
Satluj	0.109	0.121	22.73	22.73	1.273
Gomti	0.113	0.122	27.27	31.82	1.364
Ghagra	0.112	0.121	27.27	27.27	1.273
Bhagirathi	0.102	0.101	27.27	27.27	1.273
Brahmputra	0.121	0.143	27.27	27.27	1.364

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