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Evaluating genetic differentiation in wild populations of the Indian major carp, *Cirrhinus mrigala* (Hamilton–Buchanan, 1882): Evidence from allozyme and microsatellite markers

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Abstract

The population structure of *Cirrhinus mrigala* from different riverine locations in India was investigated using allozyme and microsatellite loci. *C. mrigala* samples were obtained from ten rivers belonging to Indus, Ganges, Brahmaputra and Mahanadi basins. Seven (29.2%) out of 24 allozyme loci and seven microsatellite loci were polymorphic, including five from our earlier work. Significant deviation ($P<0.05$) from the Hardy–Weinberg expectations were evident for two allozyme loci: *G6PDH**, *XDH**, and two microsatellite loci: *MFW17*, *R-12F*, in different samples. Both markers types demonstrated concordant results and various estimates revealed genetic variability within the subpopulations but surprisingly low level ($\theta=0.015$ to 0.02) of genetic differentiation among *C. mrigala* from different river samples. AMOVA analysis also indicated low differentiation among subpopulations. Common ancestry in the prehistoric period and possible exchange of individuals between rivers in different river basins such as Indus and Ganges could have been responsible for the observed low level of genetic differentiation among wild mrigal populations.

Keywords: *Cirrhinus mrigala*; Allozyme; Microsatellites; Genetic differentiation

1. Introduction

Cirrhinus mrigala or mrigal (subfamily: Cyprininae, family: Cyprinidae) is a natural inhabitant of the Indus and Ganges river systems. Natural distribution of the species ranges from Bangladesh, Nepal, and India and Pakistan. The species has been transplanted successfully from the natural range within India and to parts of Asia as well as Europe (Chondar, 1999; Froese and Pauly, 2006). The species is of commercial significance due to its aquaculture potential and high consumer preference. This fish is widely cultivated with other Indian major carps as an essential component under polyculture system because of its effective utilization of the bottom niche. According to FAO (2004), aquaculture production of *C. mrigala* was over 573,627 mt that translates to approximately 1.6% of global fish production. Wild capture fisheries of mrigal are however exhibiting a

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declining trend. Comparison of capture survey data from 1958 to 1994, indicate an 87% decline in the mrigal fishery in the middle stretch of the Ganges (Payne et al., 2004).

Stocking of Indian major carps in rivers and reservoirs is considered a favored means to augment production from capture fisheries. Cultivable fishes like *C. mrigala* can find their way to natural waters however, via accidental escape from farms. Loss of natural genetic variation is a potential risk that can result, if non-native stocks of a species are mixed. Over generations, introgression may ultimately render the species less fit to adapt to changing environments (Ferguson, 1995). Natural genetic resources also form the basis for selection of founder stocks for stock improvement programmes. Therefore, it is evident, that data from stock structure assessments can be vital for scientific planning of breeding programs aimed at conserving and maintaining wild genetic diversity. Chondar (1999) reviewed the biology and other available information on mrigal. Genetic studies on this species have been limited to karyotyping (Lakra and Krishna, 1996), Esterase polymorphism (Gopalakrishanan et al., 1997), DNA fingerprinting using Bkm and M13 probes (Majumdar et al., 1997), RAPDs (Zheng et al., 1999) and the MboI satellite (Padhi et al., 1998). Studies document genetic diversity in Asian cyprinid species (include mrigal) are important, however, in view of the multiple threats to wild cyprinid populations (Penman, 2005).

Allozyme and microsatellite markers have been used independently or collectively to document genetic diversity and to draw inference about population structure in fishes and shellfishes (Beacham et al., 2000; Ward et al., 2001; Salini et al., 2004) and to unearth population level evolution for variety of vertebrates (DeWoody and Avise, 2000; Neff and Gross, 2001; Chistiakov et al., 2006). Polymorphic allozyme (Singh et al., 2004) and microsatellite markers (Lal et al., 2004) in *C. mrigala* have been developed and these markers appear to be promising for assessing genetic differentiation in natural *C. mrigala* populations across the distribution range.

The present study assessed the natural population structure of *C. mrigala* on a macro-geographical scale in

Table 1
Sample size, location, and year of collections of *Cirrhinus mrigala* from different rivers in India

River system	River	Location	Location (lat. and log.)	Year of collection	Sample size (N)
Indus	Satluj	Heri ke patan, Amritsar, Punjab	31° 13′ N, 75° 12′ E	May–Sept., 2000	87[a]
				June, 2001	20 [a]
Ganges	Banganga	Laksar, Saharanpur, U. P.	29° 58′ N, 77° 23′ E	Dec., 2000	09 [b]
				March, 2001	05 [b]
	Ganga	Bijnore, U. P.	29° 23′ N, 79° 11′ E	May, 2002	07 [b]
		Allahabad, U. P.	25° 28′ N, 81° 54′ E	May, 2002	09 [b]
		Samaspur, U. P	26° 01′ N, 81° 03′ E	Oct.–Dec., 2000	14 [b]
	Yamuna	Etawah, U.P.	24° 47′ N, 79° 02′ E	Dec., 2000	04 [b]
		Allahabad, U. P.	25° 28′ N, 81° 54′ E	April, 2003	17 [b]
	Bhagirathi	Farraka, W. B.	24° 5′ N, 88° 06′ E	June, 2001	14 [c]
		Nabadeep, Nadia, W.B.	23° 24′ N, 88° 23′ E	Jan, 2001	50 [c]
		Beldanga, W. B.	23° 58′ N, 88° 20′ E	Jan.–May, 2001	41 [d]
		Behrampur, W. B.	24° 06′ N, 88° 19′ E	Jan.–June, 2001	61 [d]
				July, 2002	04 [d]
	Gomti	Sultanpur, U. P.	26° 16′ N, 82° 4′ E	June, 2000	69 [e]
				June, 2001	03 [e]
	Ghagara	Ajaypur, U. P.	27° 34′ N, 80° 41′ E	Dec., 2000	14 [e]
				March–Dec., 2001	68 [e]
				June, 2002	17 [e]
		Katarniya ghat, U. P.	32° 19′ N, 75° 30′ E	Dec., 2000	35 [e]
	Tons	Rewa, M. P.	24° 31′ N, 81° 17′ E	April, 2004	27 [f]
	Bhramaputra	Kalangpar, Assam	26° 11′ N, 91° 47′ E	Feb., 2000	06 [g]
				Jan., 2001	58 [g]
	Bhramaputra	Dompara, Assam	27° 28′ N, 94° 15′ E	Jan., 2001	13 [g]
Mahanadi	Mahanadi	Cuttack, Orrisa	21° 58′ N, 86° 07′ E	Jan., 2002	12 [h]
		Hirakund, Orrisa	21° 30′ N, 84° 00′ E	May, 2004	13 [h]
		Sonepur, Orrisa	21° 50′ N, 83° 56′ E	May, 2004	13 [h]
				Total	680

The common superscripts indicate the multiple data sets within rivers or neighboring localities that were pooled after testing for absence of heterogeneity.

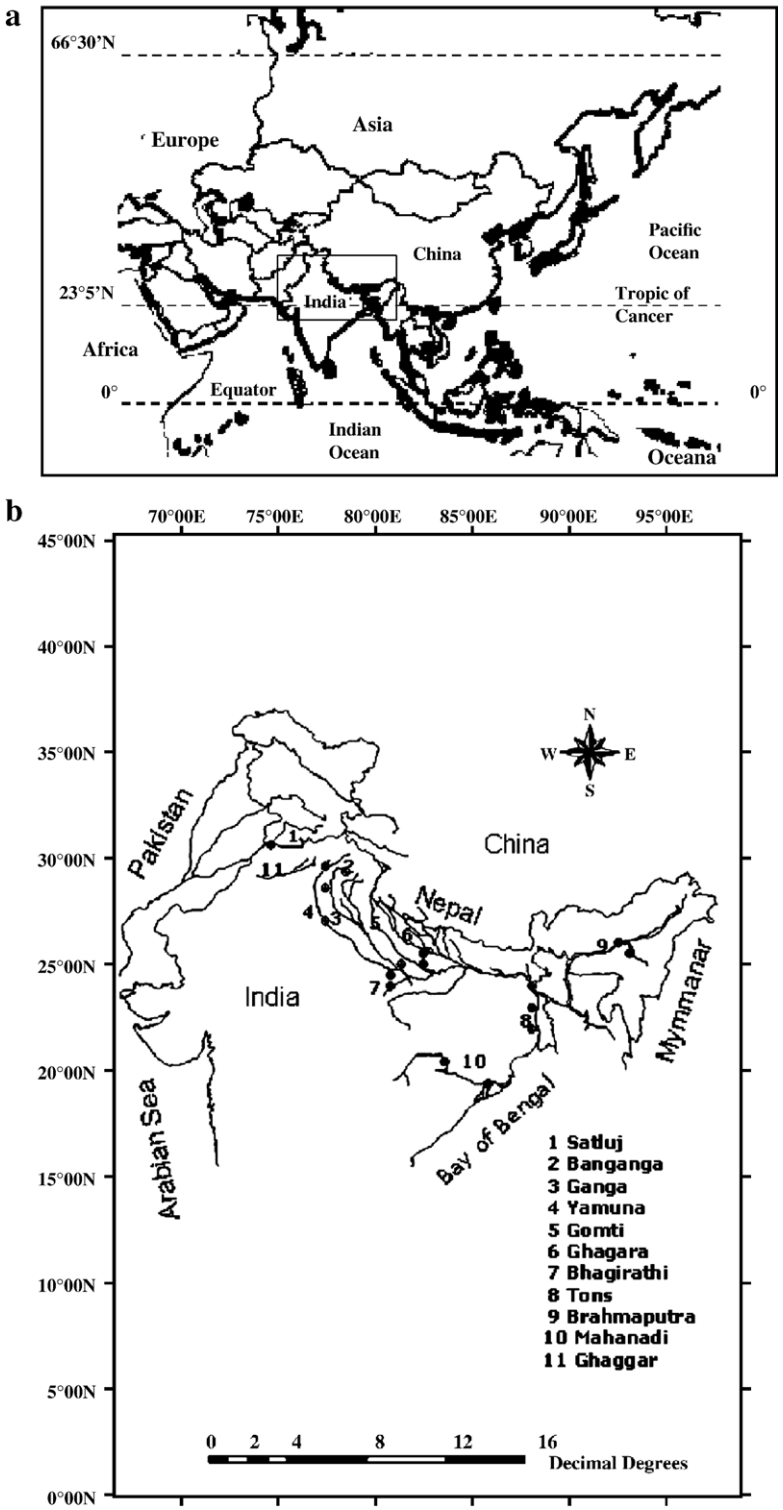

Fig. 1. (a) General map of the region, study area is located within the box. (b) Locations of sampling station (⊕) across different river basins for population structure study of *Cirrhinus mrigala*. Out of the 11 rivers listed in the figure, 10 rivers were sampled.

major rivers in India. The study used allozyme and microsatellite markers in combination. The aim of the study was to provide an assessment of genetic variation and to understand the scale of population structure across the species' major distributional range.

2. Materials and methods

2.1. Fish samples

A total 680 *C. mrigala* specimen were obtained from commercial riverine catches from different rivers between May 2000 to July 2004 (Table 1). Sampling sites were selected to document genetic variation across a wide geographical distribution range (31° 13′ N, 75° 12′E to 21° 11′N, 91° 47′E) (Fig. 1). The river Satluj belongs to the Indus basin. Other locations except for the Mahanadi R., are distant but also tributaries of the Ganges (ECAFE, 1966). Weight of specimens ranged from 1.2 to 4.5 kg. Sampling procedures were performed at actual site of collection. Liver tissue samples were taken and frozen immediately in liquid nitrogen (−196 °C). Blood was drawn by caudal puncture and fixed in 95% ethanol in 1:5 (blood: ethanol) ratio. Liver samples were then transported to the laboratory and stored at −80 °C until analysis. Blood samples were transported to the laboratory on ice and stored at 4 °C until used for genomic DNA extraction.

2.2. Allozyme analysis

Frozen liver samples (approximately 100 mg) were homogenized in 250 mg ml^{-1} extraction buffer (0.17 M Sucrose, 0.2 M EDTA, 0.2 M 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 min. Allelic variation was investigated using 7% polyacrylamide gel electrophoresis. Electrophoresis was carried out at constant voltage 150 V at 4 °C. A total 24 enzyme systems were examined and 17 enzymes yielded scorable activity (Table 2). Histochemical staining procedures outlined by Whitmore (1990) were used to visualize different alleles. Loci and alleles were designated following the nomenclature system of Shaklee et al. (1990).

2.3. Microsatellite analysis

Genomic DNA was extracted from blood via a protocol modified from Ruzzante et al. (1996), using proteinase K, and phenol: chloroform. For microsatellite analysis, five polymorphic loci *MFW1* (EF144118), *MFW2* (EF144119), *MFW17* (EF144122), *Barb54* (EF144125), *Bgon22* (EF144124) were available from our earlier work (Lal et al., 2004). To obtain more polymorphic loci, 26 primers developed for cyprinid fishes, *Labeo rohita* (AJ507518-22; AJ507524), *Cyprinus carpio* (AY169249-50; AB043469), *Camposstoma anomalum* (AF277575, 77,78, 80,82-84,88-89; AF277587) and *Pimephales promelas* (AY254350-54, 56) were examined for cross priming in *C. mrigala*. In this study, PCR amplification was performed in a 25 µl reaction mixture, that included 1X PCR buffer (10 mM Tris–HCl pH 9.0, 50 mM KCl, 0.01% gelatin), 0.2 mM of each dNTP, 2.0 mM of $MgCl_2$, 5 pmol of each primer, 1.5 U Taq DNA polymerase and 25–50 ng of template DNA. PCR (MJ PTC-200 thermal cycler) cycles were as follows (i) 1 cycle of denaturation at 94 °C for 5 min, (ii)

Table 2
The names of enzyme loci, enzyme commission (E.C.) number, observed alleles for allozyme analysis

Loci	E.C. number	Locus	Allele
Acid phosphatase	3.1.3.2	*ACP**	ns
Adenylate kinase	2.7.4.3	*AK**	100
Alcohol dehydrogenase	1.1.1.1	*ADH**	ns
Alkaline phosphate	3.1.3.1	*ALP**	ns
Aspartate amino transferase	2.6.1.1	*AAT**	78, 100
Creatine kinase	2.7.3.2	*CK**	ns
Esterase	3.1.1.1	*EST-1**	100
		*EST-2**	91, 100
Glutamate dehydrogenase	1.4.1.3	*GDH**	100
Glucose-6-phosphate dehydrogenase	1.1.1.49	*G6PDH**	94, 100, 108
Glucose phosphate isomerase	5.3.1.9	*GPI**	100, 105, 113
Glucose dehydrogenase	1.1.1.47	*GLDH**	100
Glutamate dehydrogenase	1.4.1.3	*GLUD**	ns
α-Glycerophosphate dehydrogenase	1.1.1.8	*GPDH**	82, 100
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
Malate dehydrogenase	1.1.1.37	*MDH*	100
Malic enzyme	1.1.1.40	*ME-1**	100
		*ME-2**	100
Octonol dehydrogenase	1.1.1.73	*ODH**	48, 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

ns = not scored in *Cirrhinus mrigala*.

Table 3
Characteristics of polymorphic microsatellite loci in details of *Cirrhinus mrigala*

Resource species					*C .mrigala*	
Species	Locus	Primer sequence (5′→3′)	Repeat motif	T_a (°C)	T_a (°C)	No. of alleles
Cyprinus carpio	*MFW1*	GTCCAGACTGTTCATCAGGAG GAGGTGTACACTGAGTCACGC	CA	55	57	3
	MFW 2	CACACCGGGCTACTGCAGAG GTGCAGTGCAGGCAGTTTGC	CA	55	55	5
	MFW17	CAACTACAGAGAAATTTCATC GAAATGGTACATGACCTCAAG	CA	55	51	6
Barbus barbus	*Barb54*	GTTGTTTTGATTCACACTGAG TACCATCTGCTGCTGCTTC	CA	58	50	2
Barbodes gonionotus	*Bgon22*	TCTTGTTGATCACACGGACG ACAGATGGGGAAAGAGAGCA	CCT	–	55	3
Labeo rohita	*R-3R*	TATTCACCCCAAATCCATTA GACCCTTGTGCATAAGACC	GT	–	50	2
	R-12F	CTATTCCTGTGCAGACCTTC GATACACGTCCAGTTTCACC	AC	–	55	4

25 cycles of denaturation at 94 °C for 30 s, relevant annealing temperature for 30 s, elongation at 72 °C for 1 min, (iii) a final elongation of 1 cycle at 72 °C for 4 min and stored at 4 °C. PCR products were resolved through vertical non-denaturing polyacrylamide (19:1 acrylamide: bisacrylamide) gels electrophoresis (size 10×10.5 cm, Amersham Biosciences Ltd.). Electrophoresis was done with 1X TBE buffer for 5 h at 10 v/cm at 4 °C. Gel concentrations and annealing temperatures (Table 4) were optimized to obtain clear scorable allelic banding patterns. Amplified microsatellite loci were visualized via silver staining (silver staining kit, Amersham Biosciences, USA). Alleles were designated according to PCR product size, calculated relative to a

Table 4
Alleles and allele frequencies at seven polymorphic allozyme loci in *Cirrhinus mrigala* from eight different riverine locations

Locus/ allele	Populations							
	Satluj	Ganga	Bhagirathi	Gomti	Ghagara	Tons	Brahmaputra	Mahanadi
(*n*)	94	49	156	69	100	26	50	33
*AAT**								
78	0.0000	0.0200	0.0000	0.0294	0.0106	0.0000	0.0000	0.0000
100	1.0000	0.9800	1.0000	0.9706	0.9894	1.0000	1.0000	1.0000
*EST-2**								
91	0.4149	0.5196	0.5481	0.4058	0.5663	0.5385	0.5100	0.6857
100	0.5851	0.4804	0.4519	0.5942	0.4337	0.4615	0.4900	0.3143
*G6PDH**								
94	0.3172	0.4100	0.2981	0.4710	0.3711	0.1667	0.2600	0.4286
100	0.4516	0.4600	0.6603	0.4855	0.5155	0.6042	0.6600	0.4857
108	0.2312	0.1300	0.0417	0.0435	0.1134	0.2292	0.0800	0.0857
*GPDH**								
82	0.2239	0.2667	0.1364	0.2203	0.2216	0.2750	0.1932	0.1538
100	0.7761	0.7333	0.8636	0.7797	0.7784	0.7250	0.8068	0.8462
*GPI**								
100	0.6064	0.5294	0.6314	0.5507	0.5206	0.5185	0.6400	0.4571
105	0.0000	0.0490	0.0032	0.0362	0.0464	0.0370	0.0200	0.0000
113	0.3936	0.4216	0.3654	0.4130	0.4330	0.4444	0.3400	0.5429
*ODH**								
48	0.1141	0.0600	0.1186	0.0441	0.1082	0.1200	0.1500	0.1029
100	0.8859	0.9400	0.8814	0.9559	0.8918	0.8800	0.8500	0.8971
*XDH**								
93	0.4830	0.4783	0.4866	0.4000	0.4255	0.5192	0.3404	0.5625
100	0.5170	0.5217	0.5134	0.6000	0.5745	0.4808	0.6596	0.4375

Numbers of specimens (*n*) are given for each sample.

molecular marker (pBR322 DNA/MspI digest) with Image master 1D Elite v3.01 (Amersham Biosciences, USA). A non-denaturing electrophoresis system has been found to provide the same resolution as that obtained with denaturing acrylamide gels and silver staining with the additional advantage of ease of use for analysing large sample sizes (Wang et al., 2003). Moreover, Bovo et al. (1999) demonstrated that non-denaturing electrophoresis is not responsible for spurious or multiple bands in microsatellite analysis.

2.4. Data analysis

Individual fish genotypes at each allozyme and microsatellite locus were determined These data were then analyzed for homogeneity between data sets for collections at different times and neighboring localities within each river. Data sets within each river or neighboring tributaries that were not heterogenous ($P>0.05$) were later combined for further analysis for estimating genetic variation and differentiation parameters. A locus was considered polymorphic, if the frequency of the most common allele was less than or equal to 0.99 (Hartl and Clark, 1997). Allele frequencies and heterozygosity (observed and expected) values were calculated using Genetix *ver.* 4.05 software (Belkhir et al., 1997). Tests for conformity to Hardy–Weinberg expectations (probability test) and linkage disequilibrium were undertaken in Genepop *ver.*3.3d software (Raymond and Rousset, 1995a). Genetic heterogeneity of all population and pairwise localities were determined using an exact test (*G* based test) that assumes random samples of genotypes (Genepop *ver.* 3.3d, Genotype differentiation test, Raymond and Rousset, 1995a). This

Table 5
Allele size (bp) and allele frequencies at seven microsatellite loci in *Cirrhinus mrigala* from different riverine locations

Locus/ allele	Populations							
	Satluj	Ganga	Bhagirathi	Gomti	Ghagara	Tons	Brahmaputra	Mahanadi
MFW1								
(*n*)	97	58	159	66	140	27	61	38
168	0.6477	0.6667	0.5654	0.6774	0.6653	0.5370	0.6429	0.5263
170	0.3523	0.3254	0.4346	0.3145	0.3347	0.4630	0.3571	0.4737
172	0.0000	0.0079	0.0000	0.0081	0.0000	0.0000	0.0000	0.0000
MFW2								
162	0.0000	0.0000	0.0000	0.0085	0.0000	0.0000	0.0091	0.0000
166	0.5111	0.6316	0.6987	0.6017	0.5966	0.6957	0.6455	0.6029
170	0.4222	0.2982	0.2450	0.3475	0.3739	0.3043	0.2909	0.3235
172	0.0667	0.0526	0.0563	0.0424	0.0252	0.0000	0.0545	0.0735
176	0.0000	0.0175	0.0000	0.0000	0.0042	0.0000	0.0000	0.0000
MFW17								
210	0.0427	0.0204	0.0481	0.0700	0.0275	0.0000	0.0270	0.0714
216	0.0122	0.0510	0.0222	0.0200	0.0165	0.0682	0.0135	0.0000
218	0.5244	0.2959	0.3889	0.3300	0.4780	0.4545	0.4730	0.6071
220	0.1829	0.3776	0.3407	0.3400	0.2473	0.3636	0.2432	0.1786
226	0.0671	0.1633	0.0926	0.0800	0.1099	0.0682	0.0811	0.1071
228	0.1707	0.0918	0.1074	0.1600	0.1209	0.0455	0.1622	0.0357
Bgon22								
110	0.5170	0.5308	0.6833	0.5000	0.4652	0.4800	0.5965	0.5294
113	0.4773	0.4692	0.3167	0.4922	0.5348	0.5200	0.4035	0.4706
116	0.0057	0.0000	0.0000	0.0078	0.0000	0.0000	0.0000	0.0000
Barb54								
87	0.9950	1.0000	1.0000	1.0000	1.0000	1.0000	0.9900	1.0000
89	0.0050	0.0000	0.0000	0.0000	0.0000	0.0000	0.0100	0.0000
R-12F								
130	0.1739	0.1695	0.2333	0.2295	0.1447	0.2400	0.1404	0.1618
134	0.0109	0.0169	0.0100	0.0164	0.0044	0.0000	0.0175	0.0294
136	0.5054	0.4831	0.5167	0.4426	0.5614	0.6000	0.5965	0.5294
138	0.3098	0.3305	0.2400	0.3115	0.2895	0.1600	0.2456	0.2794
R-3R								
103	0.9950	1.0000	1.0000	0.9900	1.0000	0.9820	1.0000	1.0000
105	0.0050	0.0000	0.0000	0.0100	0.0000	0.0180	0.0000	0.0000

Numbers of specimens (*n*) are given for each sample.

test is performed on genotype tables and possible non-independence of alleles within genotypes does not affect test validity (Raymond and Rousset, 1995b; Goudet et al., 1996). The null hypothesis tested was, that the genotype distribution was identical across all populations. Fixation indices based on an infinite allele model (IAM, Kimura and Crow, 1964) and a stepwise-mutation model (SMM, Kimura and Ohta, 1978) were estimated to determine the extent of population subdivision among samples. For the former, Genetix *ver*. 4.05 software (Belkhir et al., 1997) was used to estimate *F*-statistics (Wright, 1951) computed as estimatorsθ, F and f of Weir and Cockerham (1984). Probability of θ significantly deviating from zero was calculated using 1000 bootstraps. Under a SMM, model, estimates of Rst (Slatkin, 1995) were made using the Genepop *ver*. 3.3d software). To correct for multiple simultaneous comparisons, sequential Bonferroni corrections were applied using a global significance level of 0.05 (Lessios, 1992). Microsatellite genotype data were checked for possible null alleles using the software FreeNA (Chapuis and Estoup, 2007) and F_{st} values

Table 6
Parameters of genetic variation for the seven polymorphic loci in *Cirrhinus mrigala* from eight different riverine locations

Locus	Populations							
	Satluj	Ganga	Bhagirathi	Gomti	Ghagara	Tons	Brahmaputra	Mahanadi
*AAT**								
H_o	0.0000	0.0400	0.0000	0.0588	0.0213	0.0000	0.0000	0.0000
H_e	0.0000	0.0392	0.0000	0.0571	0.0211	0.0000	0.0000	0.0000
F_{is}	–	−.010	–	−.023	−.005	–	–	–
P_{HW}	–	1.0000	–	1.0000	1.0000	–	–	–
*EST-2**								
H_o	0.5106	0.4118	0.4679	0.4928	0.4592	0.5385	0.4200	0.4000
H_e	0.4855	0.4992	0.4954	0.4823	0.4912	0.4970	0.4998	0.4310
F_{is}	−0.046	+0.185	+0.059	−0.014	+0.070	−0.064	+0.169	+0.086
P_{HW}	0.6760	0.2609	0.5186	1.0000	0.5395	1.0000	0.2661	0.6997
*G6PDH**								
H_o	0.5806	0.6800	0.4615	0.8261	0.5773	0.3750	0.5600	0.4571
H_e	0.6420	0.6034	0.4735	0.5405	0.5837	0.5547	0.4904	0.5731
F_{is}	+0.101	−0.117	+0.028	−0.523	+0.016	+0.343	−0.132	+0.216
P_{HW}	<0.0001**	0.0003**	<0.0001**	<0.0001**	<0.0001**	0.0002**	0.0850	0.0111*
*GPDH**								
H_o	0.2985	0.4444	0.2308	0.3729	0.3977	0.2500	0.2500	0.3077
H_e	0.3475	0.3911	0.2355	0.3436	0.3450	0.3987	0.3117	0.2604
F_{is}	+0.148	−0.125	+0.024	−0.077	−0.147	+0.395	+0.209	−0.163
P_{HW}	0.2876	0.4721	0.7257	0.7132	0.2209	0.1072	0.1744	1.0000
*GPI**								
H_o	0.4468	0.6078	0.4679	0.5217	0.4227	0.4815	0.6400	0.5143
H_e	0.4774	0.5396	0.4678	0.5248	0.5393	0.5322	0.4744	0.4963
F_{is}	+0.069	−0.117	+0.003	+0.013	+0.221	+0.114	−0.340	−0.022
P_{HW}	0.5228	0.5634	1.0000	0.3377	0.0016*	0.8447	0.0301*	1.0000
*ODH**								
H_o	0.1848	0.0800	0.1859	0.0882	0.1753	0.2400	0.3000	0.2059
H_e	0.2022	0.1128	0.2091	0.0843	0.1931	0.2112	0.2550	0.1847
F_{is}	+0.092	+0.300	+0.114	−0.039	+0.097	−0.116	−0.167	−0.100
P_{HW}	0.3218	0.1468	0.2346	1.0000	0.2974	1.0000	0.5754	1.0000
*XDH**								
H_o	0.5341	0.6522	0.651	0.6154	0.7234	0.5769	0.4681	0.4375
H_e	0.4994	0.4991	0.4996	0.4800	0.4889	0.4993	0.4491	0.4922
F_{is}	−0.064	−0.297	−0.300	−0.275	−0.476	−0.136	−0.032	+0.127
P_{HW}	0.6691	0.0741	0.0003**	0.0379*	<0.0001**	0.6938	1.0000	1.0000
Mean over all loci								
H_o	0.1162	0.1326	0.1121	0.1353	0.1262	0.1119	0.1199	0.1056
H_e	0.1206	0.1220	0.1082	0.1142	0.1210	0.1224	0.1127	0.1108
$P_{0.95}$	0.2727	0.2727	0.2727	0.2273	0.2727	0.2727	0.2727	0.2727
$P_{0.99}$	0.2727	0.3182	0.2727	0.3182	0.3182	0.2727	0.2727	0.2727
A_n	1.3182	1.4091	1.3636	1.4091	1.4091	1.3636	1.3636	1.3182

* Significant values ($P<0.05$), ** Significant after Bonferroni adjustment.

were computed using both corrected and uncorrected data. The hierarchical analysis was carried out using analysis of molecular variance (AMOVA) in the Arlequin 2000 package (Excoffier et al., 2005). AMOVA yields estimations of population structure at different levels of the specified hierarchy using three levels of population structure: within population, among subpopulations within river basins and among river basins. For the analysis, different subpopulations were grouped as per three unlinked river basins viz. Indus, Ganga and Mahanadi (Table 1).

3. Results

3.1. Genetic variation

Of 24 allozyme loci examined, seven loci — *AAT*, EST-2*, G6PDH**, *GPDH**, *GPI**, *ODH** and *XDH**, were polymorphic (29.2%) in *C. mrigala* (Table 2). Out of 26 microsatellite primers from four cyprinid fishes, six primer pairs yielded amplified products. Two microsatellite loci — *R-3R* (EF144126), (EF144127), *R-12F* were polymorphic. Therefore, a total of seven polymorphic

Table 7
Parameters of genetic variation at seven microsatellite loci in *Cirrhinus mrigala* from different riverine locations

Locus	Populations							
	Satluj	Ganga	Bhagirathi	Gomti	Ghagara	Tons	Brahmaputra	Mahanadi
MFW1								
H_o	0.5455	0.3651	0.4379	0.4516	0.4831	0.4074	0.4286	0.5789
H_e	0.4564	0.4496	0.4915	0.4421	0.4454	0.4973	0.4592	0.4986
F_{is}	+0.190	+0.196	+0.112	−0.013	−0.080	+0.199	+0.077	−0.148
P_{HW}	0.1011	0.0791	0.1890	1.0000	0.4145	0.4388	0.7551	0.5140
MFW2								
H_o	0.5111	0.4737	0.4238	0.4576	0.5126	0.4348	0.3636	0.5588
H_e	0.5560	0.5091	0.4486	0.5154	0.5035	0.4234	0.4957	0.5264
F_{is}	+0.086	+0.078	+0.059	+0.120	−0.014	−0.005	+0.275	−0.047
P_{HW}	0.4671	0.1159	0.5067	0.4099	0.0910	1.0000	0.0148*	0.0710
MFW17								
H_o	0.5244	0.5306	0.4667	0.6200	0.5055	0.3636	0.5405	0.3214
H_e	0.6559	0.7318	0.7097	0.7382	0.6826	0.6498	0.6833	0.5816
F_{is}	+0.206	+0.284	+0.346	+0.170	+0.265	+0.459	+0.222	+0.462
P_{HW}	<0.0001**	0.0021*	<0.0001**	0.0019*	<0.0001**	0.0008**	0.1114	0.0008**
Bgon22								
H_o	0.5795	0.5077	0.3800	0.5313	0.5130	0.4800	0.4211	0.4118
H_e	0.5048	0.4981	0.4328	0.5077	0.4976	0.4992	0.4814	0.4983
F_{is}	−0.142	−0.011	+0.125	−0.039	−0.027	+0.059	+0.134	+0.188
P_{HW}	0.1992	1.0000	0.1342	0.8025	0.8517	1.0000	0.4085	0.3167
Barb54								
H_o	0.0104	0.0000	0.0000	0.0000	0.0000	0.0000	0.0182	0.0000
H_e	0.0104	0.0000	0.0000	0.0000	0.0000	0.0000	0.0180	0.0000
F_{is}	–	–	–	–	–	–	–	–
P_{HW}	–	–	–	–	–	–	–	–
R-12F								
H_o	0.5978	0.5085	0.5400	0.5902	0.4035	0.6000	0.4386	0.4706
H_e	0.6182	0.6284	0.6209	0.6541	0.5801	0.5568	0.5639	0.6146
F_{is}	+0.038	+0.199	+0.134	+0.106	+0.308	−0.057	+0.231	+0.248
P_{HW}	0.0455*	0.0195*	0.0037*	0.7292	0.0000**	0.6038	0.0044*	0.0060*
R-3R								
H_o	0.0103	0.0000	0.0000	0.0152	0.0000	0.0370	0.0000	0.0000
H_e	0.0103	0.0000	0.0000	0.0150	0.0000	0.0364	0.0000	0.0000
F_{is}	–	–	–	–	–	–	–	–
P_{HW}	–	–	–	–	–	–	–	–
Mean over all loci								
H_o	0.4040	0.4060	0.3875	0.4139	0.3888	0.3884	0.3899	0.3945
H_e	0.4017	0.4024	0.3862	0.4104	0.3870	0.3804	0.3859	0.3885
$P_{0.95}$	0.7143	0.7143	0.7143	0.7143	0.7143	0.7143	0.7143	0.7143
$P_{0.99}$	0.7143	0.7143	0.7143	0.7143	0.7143	0.8571	0.7143	0.7143
A_n	3.1429	3.0000	2.7143	3.2857	2.8571	2.4286	3.0000	2.5714

* Significant values ($P<0.05$), ** Significant after Bonferroni adjustment.

Table 8
Fisher's Exact test of allozyme and microsatellite allele homogeneity for all the populations pairs of *Cirrhinus mrigala*

	Significant allelic homogeneity		All over loci (*P*-exact)	
Population pairs	Allozyme loci	Microsatellite loci	Allozyme	Microsatellite
Ganga and Satluj	*GPI*	*MFW2, MFW17***	0.01255*	0.03981*
Bhagirathi and Satluj	*EST2, G6PDH**, GPDH*	*MFW2**, *MFW17, Bgon22***	<0.0001**	<0.0001**
Bhagirathi and Ganga	*G6PDH**, *GPDH, GPI***	*MFW1, Bgon22*	0.00005**	0.00225*
Gomti and Satluj	*AAT, G6PDH**, *GPI, ODH*	*MFW17*	0.00001**	0.59757
Gomti and Ganga	–	–	0.27857	0.80179
Gomti and Bhagirathi	*AAT, EST2, G6PDH, GPI, ODH*	*MFW1, Bgon22***	<0.0001**	0.00160**
Ghagara and Satluj	*EST2, G6PDH, GPI*	–	0.00231**	0.44578
Ghagara and Ganga	–	–	0.74953	0.22208
Ghagara and Bhagirathi	*G6PDH, GPDH, GPI***	*MFW1, MFW2, Bgon22***	0.00014**	<0.0001**
Ghagara and Gomti	*EST2, G6PDH, ODH*	–	0.02996*	0.26775
Tons and Satluj	*GPI*	*MFW2, MFW17*	0.16651	0.04897*
Tons and Ganga	*G6PDH*	–	0.60885	0.17092
Tons and Bhagirathi	*G6PDH**, *GPI*	*Bgon22*	0.01057*	0.053
Tons and Gomti	*G6PDH*	–	0.00148**	0.16279
Tons and Ghagara	*G6PDH*	–	0.50187	0.1416
Brahmaputra and Satluj	*G6PDH**, *XDH*	–	0.00214**	0.84568
Brahmaputra and Ganga	*G6PDH*	–	0.02285*	0.53523
Brahmaputra and Bhagirathi	*XDH*	–	0.07403	0.23032
Brahmaputra and Gomti	*G6PDH**, *ODH*	–	0.00176**	0.75725
Brahmaputra and Ghagara	–	*Bgon22*	0.17424	0.3059
Brahmaputra and Tons	*XDH*	–	0.17182	0.34824
Mahanadi and Satluj	*EST2**, *GPI*, *G6PDH*	–	<0.0001**	0.65804
Mahanadi and Ganga	*GPI*	*MFW17*	0.00015**	0.07507
Mahanadi and Bhagirathi	*GPI*	*MFW17, Bgon22*	<0.0001**	0.07223
Mahanadi and Gomti	*EST2**, *GPI, XDH*	*MFW1, MFW17*	<0.0001**	0.16749
Mahanadi and Ghagara	*XDH*	*MFW1*	<0.0001**	0.15251
Mahanadi and Tons	*G6PDH*	–	0.00027**	0.32737
Mahanadi and Brahmaputra	*EST-2, GPI, XDH*	–	<0.0001**	0.70055

* Significant at $P<0.05$, ** Significant after sequential Bonferroni adjustment.

microsatellite loci (five from our earlier work) were available to genotype *C. mrigala* samples from different riverine localities (Table 3). No significant genotype heterogeneity was observed between the multiple data sets (collections at different time intervals and neighboring locations) within the rivers Ganga (Banganga, Ganga and Yamuna), Bhagirathi and Ghagara (Table 1). After combining the genotypic data from multiple data sets within each river, eight data sets for the rivers, Satluj, Ganga, Bhagirathi, Gomti, Ghagara, Tons, Brahmaputra and Mahanadi were available for analysis of genetic variation and differentiation among *C. mrigala* populations. Allele frequencies at polymorphic allozyme and microsatellite loci in *C. mrigala* samples from the eight localities are presented in Tables 4 and 5, respectively. No population-specific alleles were observed for any allozyme

Table 9
Pairwise F_{st} between riverine samples of *Cirrhinus mrigala* using allozyme loci

F_{st}	Ganga	Bhagirathi	Gomti	Ghagara	Tons	Brahmaputra	Mahanadi
Satluj	0.00325	0.02164**	0.01088	0.01029**	0.00400	0.01783	0.03528**
Ganga		0.01749**	0.00300	−0.00285	0.00381	0.01891	0.01127
Bhagirathi			0.02579**	0.01046**	0.01218	0.00546	0.02405**
Gomti				0.00925	0.03024**	0.02039**	0.0394**
Ghagara					0.00384	0.00857	0.00873
Tons						0.01163**	0.01800
Brahmaputra							0.04557**

** Significant after sequential Bonferroni adjustment ($P<0.00416$).

Table 10
Pairwise F_{st} (above diagonal) and Rst (below diagonal) between riverine samples of *Cirrhinus mrigala* using microsatellite loci

F_{st}	Satluj	Ganga	Bhagirathi	Gomti	Ghagara	Tons	Brahmaputra	Mahanadi
Satluj	–	0.01669**	0.02860**	0.00785	0.00050	0.01687	0.00348	0.00270
Ganga	−0.0022	–	0.01209	−0.0048	0.00686	0.00665	0.00561	0.02051
Bhagirathi	0.0120**	0.0105	–	0.01616**	0.02647**	0.00765	0.00423	0.01690.
Gomti	−0.0011	−0.0050	0.0035	–	0.00372	0.00832	0.00580	0.01863
Ghagara	−0.0020	−0.0041	0.0152**	−0.0021	–	0.00435	0.00098	0.00423
Tons	0.0137	0.0200	−0.0020	0.0019	0.0153	–	0.00093	0.00011
Brahmaputra	−0.0011	−0.0083	0.0017	−0.0054	−0.0034	0.0108	–	0.00075
Mahanadi	0.0121	0.0290	0.0183	0.0196	0.0177	0.0023	0.0197	–

** Significant after sequential Bonferroni adjustment ($P<0.0025$).

or microsatellite locus. Five microsatellite loci, *MFW1*, *2*, *17*, *Bgon22*, *R-12F* exhibited considerable variation in all the sampled populations. Two loci *Barb54* and *R-3R* were found to possess rare alleles in low frequency (equal or less than 0.01) in specific populations.

Summary statistics for parameters of genetic variation at each allozyme and microsatellite locus and across all loci are given in Tables 6 and 7, respectively. Mean number of alleles per locus ranged from 1.32 to 1.41 for allozyme loci and 2.29 to 3.29 for microsatellite loci. Mean values of observed heterozygosity ranged from 0.11 to 0.14 for allozyme loci and from 0.39 to 0.40 for microsatellite loci respectively.

Two allozyme loci *G6PDH**, *XDH** (Table 6) and two microsatellite loci, *MFW17*, *R-12F* (Table 8), exhibited consistent significant deviations from H–W equilibrium expectations in some samples, after the probability level ($P=0.05$) was adjusted for sequential Bonferroni correction. Significant deviation at the *G6PDH** locus was found in samples from all localities except for the Brahmaputra and Mahanadi and at locus *XDH** only in the Bhagirathi and Ghagara samples. For microsatellite loci, significant deviation from HW expectations was evident (Table 7) in the Satluj, Bhagirathi, Ghagara, Tons and Mahanadi populations at locus MFW17. F_{is} values greater than zero (+ve), indicating deficiency of heterozygotes or lower than zero (−ve) indicating an excess of heterozygotes, were evident in these cases (Tables 6 and 7). No test for linkage disequilibrium was statistically significant ($P>0.05$) for any pair of allozyme or microsatellite loci within each of the sample sites and when all samples were considered together.

Table 11
Analysis of molecular variance (AMOVA) based on microsatellite and allozyme (values given in parenthesis) alleles in *C. mrigala* population

Sources of variation	Variance component	Percentage of variation (%)	Fixation indices
Among river	−0.0001	−0.01	−0.0023
Basins	(0.0012)	(0.02)	(−0.005)
Among subpopulations	−0.0047	−0.023	−0.0023
Within river basins	(−0.0367)	(−0.056)	(−0.0056)
Within subpopulations	2.0988	100.23	−0.0006
	(6.6215)	(100.54)	(0.0002)

3.2. Population structure

An exact test for population differentiation was performed to assess homogeneity for 28 possible pairs of sample localities at each allozyme and microsatellite locus. In pairwise comparisons, significant probabilities ($P<0.05$) at least at one locus, were found for nine pairs (allozyme) and four pairs (microsatellites) of sample localities (Table 8) after sequential Bonferroni adjustment. A *G*-test for genotype homogeneity, across all loci showed that 16 pairs (allozyme) and three pairs (microsatellites) were significantly different, when levels were adjusted for sequential Bonferroni correction (Table 8).

Fixation indices under an IAM model (F_{st}) were consistent for the two marker types. The mean F_{st} value across all populations and all loci was 0.020 (allozyme) and 0.013 (microsatellite). After correction for possible null alleles in the microsatellite data, mean F_{st} value across all populations and all loci was 0.0127. Pairwise comparisons of F_{st}, with probabilities of significance are given in Tables 9 (allozyme) and 10 (microsatellite). Fixation indices under SMM model (Rst) were found to be comparable with F_{st} values in pairwise comparisons of samples. AMOVA analysis revealed that 100% of the variance was explained by within subpopulation variation (Table 11). There was evidence of only weak differentiation among different *C. mrigala* subpopulations sampled from rivers belonging to three river basins.

4. Discussion

The present study reports the distribution and patterns of genetic variation in natural populations of *C. mrigala* estimated from allozyme and microsatellite markers. Ruzzante (1998) demonstrated that sample sizes larger than 50 individuals are adequate to minimize bias due to large number of alleles in microsatellite data and Silva and Russo (2000) inferred that sample size should be more than 30. In the present study, samples sizes were greater than 50 individuals per site in six of eight localities analyzed. Therefore, estimates of population differentiation obtained, are unlikely to be confounded by small sample sizes.

For allozyme loci, genetic variability in *C. mrigala* was relatively high (H_o: 0.105–0.135) when compared with values described for many freshwater fish species (Gyllensten, 1985 — 0.043; Ward et al., 1994 — 0.046). Observed heterozygosity values were also relatively high and within the range reported for teleost fish species (0.05–0.07) described by Nevo (1978). As reported for several vertebrates (Nevo et al., 1984) and plants (Frankham, 1996), populations of widespread species often show significantly higher heterozygosity estimates than for populations of species with more restricted distribution. Among European cyprinids, the common, wide spread and opportunistic roach *Rutilus rutilus* (L.) exhibits a high degree of variability (H_e=0.097–0.124; Bouvet et al., 1991) in contrast to the endemic and rare *Leuciscus* species (H_e=0.000–0.057) and *Chondrostoma* species (H_e=0.022–0.070) (Coelho, 1992; Alves and Coehlo, 1994; Coelho et al., 1995). Genetic variability estimates for *C. mrigala* (heterozygosity 0.38–0.42; alleles per locus 2–7) for microsatellite loci closely approximate values reported for most freshwater fishes (heterozygosity, 0.54±0.25; alleles per locus 9.1±6.1; DeWoody and Avise, 2000).

Deviations from Hardy–Weinberg genotypic expectations were observed at some allozyme and microsatellite loci here. Several possible alternative explanations may explain these observations. First, microsatellite loci may possess null alleles that do not amplify, producing heterozygotes that cannot be distinguished (Paetkau and Strobeck, 1995; Ishibashi et al., 1997). No evidence of null allele homozygotes were detected however, in any of the populations analyzed. This indicates that null alleles, are absent at these loci or were not in significant frequency to be a major cause of the observed heterozygote deficits. Moreover, genetic differentiation observed after correction for possible null alleles did not differ from that obtained with uncorrected data. Where homozygote excesses were detected, generally such deviations indicate that factors such as non-random mating, reduction in effective breeding population or specific locus could be under selection pressure were the causes for the observed violations. (Ferguson, 1995; Garcia de Leon et al., 1997). Heterozygote deficits can also result from mixing of undetected genetically divergent stocks within the samples, referred to as Wahlund effect (Hartl and Clark, 1997). With respect to *C. mrigala*, fish escaping to rivers could have given rise to such a situation if gene frequencies in farmed stocks diverged from wild populations where they were sampled. Generally, farmed or hatchery reared fish tend to have different genetic backgrounds compared with their wild conspecifics and are also likely to possess lower genetic diversity. Eknath and Doyle (1990) have reported loss of genetic variation due to inbreeding in Indian major carp hatcheries in the past. Undetected presence of hatchery-reared escapees in the samples could explain the underestimate of the heterozygotes in the data here. The observed excess of heterozygotes at the *G6PDH** and *XDH** loci in some samples may also have resulted from outbreeding of wild fish with non-native farmed escapes. While, the present analysis did not pinpoint evidence for presence of farm escapes among the samples, if present, they could be a possible threat to wild germplasm.

Comparable F_{st} (θ) estimates from allozyme (0.020) and microsatellite (0.013) loci clearly indicate that wild *C. mrigala* populations are only weakly sub structured and that only 1.5 to 2.0% of the total observed genetic variation resulted from population differentiation. Wright (1978) and Hartl and Clark (1997) suggested that F_{st} estimates in the range 0–0.05 indicate little genetic differentiation among populations. Ward et al. (1994) reviewed 49 freshwater fish species and observed F_{st} estimates ranged from 0 to 74% with a mean of 22.2%. In this survey, 23 freshwater fish species out of 49 species had genetic differentiation (F_{st}) ranging from 0 to 10% (Ward et al., 1994). AMOVA analysis of the data also did not indicate any significant genetic differentiation among sampled populations.

Genetic differentiation can be influenced by a number of evolutionary forces and their interaction that act on natural populations including; migration, random genetic drift, mutation etc. (Hartl and Clark, 1997). Random genetic drift will tend to cause genetic differentiation, after subpopulations are fragmented and gene flow between them is either reduced or absent. Paleogeographical reconstructions clearly identify the possibility that *C. mrigala* from different river basins sampled here, are likely to have evolved from common

ancestral gene pool. The genus *Cirrhinus* is considered to have entered India during the Eocene following migration of Indo-Malayan fishes via the Indo-Brahma River, flowing westward from Assam in the north-east to the present-day Arabian Sea (Daniel, 2001). Migration of fishes that evolved during the Eocene (60 million years ago) continued until dismemberment of the Indo-Brahma River and formation of the Indus, Ganga and Brahmaputra river systems during the late Pleistocene. Modern rivers such as the Satluj, Beas, Yamuna, Ghagara and other Himalayan rivers were formed as lateral rivers to the Ganges more recently.

In spite of fragmentation, low genetic divergence among wild mrigal populations may result from extensive ongoing gene flow among populations in the Indus and Ganges river systems via connectivity across common flood plains and changes in the course of associated rivers. Remote sensing and archaeological evidence suggests that the seasonal Ghaggar river basin (Fig. 1) located between the Indus and Ganga basins is the remnant of the ancient mighty perennial Saraswati river with the present day Satluj river as its northwest tributary (Puri and Verma, 1998; Lal, 2002). A hypothesised change in the course of the Satluj R. to join the Indus system that occurred around 1900 BC (Lal, 2002) may have resulted in large scale mixing of putative subpopulations of aquatic organisms in the Saraswati R and Indus river systems. North-eastern tributaries and rivulets of the Ghaggar R. (erstwhile Saraswati) over the years have been flowing close to the Yamuna River (a tributary of the Ganga). Nevertheless, it is likely that the tributaries that belong to different river basins including the Indus, Ganges and the intermediary Ghaggar basin that still flow in close proximity could be the source of mixing of fish populations during floods. Such periodical gene flow could offset any divergence that random genetic drift might possibly cause when conditions are drier (Hartl and Clark, 1997). Besides direct migration, a stepping stone model of migration that attributes effective gene flow to gene exchange among neighbors (Felsenstein, 1997) may also explain the lack of significant allelic heterogeneity, among mrigal population in the river systems sampled here. The observed lack of private or locality specific allele at any of allozyme or microsatellite loci argues in favor of effective ongoing gene flow. Therefore, common ancestry in the past and possible recent intermittent exchange of individuals among rivers belonging to different river basins may explain the observed low levels of genetic differentiation among mrigal populations. Comparable values for fixation indices based on the SMM (RST) and IAM (θ) estimates for microsatellite data, indicate that the observed genetic structure of *C. mrigala* population is likely to be of recent origin. In effect, there may have been insufficient time for isolation and mutational events to give rise to new alleles and unique genotypes.

The four associated rivers of the Ganges, viz. Ganga main channel, Gomti, Ghagara and Tons appear to share a common gene pool of *C. mrigala*. This is possible via connections associated with a common flood plain and likely dispersal of fish from the Ganga main channel to these tributaries. The observed small differentiation (F_{st}) between the Satluj R. and two localities on the Ganges viz. Ganga (0.0167, microsatellite $P=0.001$) and Ghaggra (0.00103, allozyme, $P=0.002$) were not significant when population pairs were compared after the loci *G6PDH**, (allozyme) and *MFW17* (microsatellite) loci were excluded from the analysis. Both these loci exhibited significant heterozygote deficiencies in the samples. Therefore, the small genetic differentiation evident here contributed by variation at these two loci may not be conclusive evidence for considering the Satluj R. population as a distinct genetic stock from the *C. mrigala* in the Ganga and Ghaggra rivers.

No significant differentiation was evident between the Brahmaputra samples when compared with samples from any of the other seven localities including the Satluj river. The Brahmaputra joins the main Ganga channel (Padma River) as the Jamuna River (ECAFE, 1966). Significant genetic divergence was evident between the Brahmaputra samples from Gomti and the Satluj samples only for allozyme loci but was not found significant after exclusion of the *G6PDH** locus. One interesting observation was that the Bhagirathi samples exhibited significant divergence from other localities in the Ganges in addition to the Satluj. This evidence from both marker types was obtained from allelic heterogeneity over all loci and fixation indices (F_{st} and Rst), which deviated significantly from zero even after exclusion of the *G6PDH**, *XDH** and *MFW17* loci. This unexpected outcome requires further comment. The Bhagirathi–Hooghly drainage is the most western stretch of the Ganga delta. The river was the main channel of the Ganges until the river changed course in the 15th century leading to silting and disconnecting of the Bhagirathi–Hooghly (ECAFE, 1966; Bhattacharya, 1973). The river was rejuvenated via a feeder canal only in 1975. It is likely that the alteration of allele frequencies and genetic differentiation of mrigal populations in the Bhagirathi occurred during this period of restricted migration. Recent reproductive mixing with farm escapees could be an

alternative explanation for the observed changes in allele frequency. The Bhagirathi–Hooghly drainage traverses across the state of West Bengal, a hub for IMC seed production and aquaculture in India. Moreover, the observed excess of heterozygotes at the *G6PDH** and *XDH** loci in Bhagirathi samples support the possibility that allele frequencies could have been affected, due to mixing of escaped farmed fish.

Evidence for divergence of the Mahanadi samples from the Satluj, Gomti, Brahmaputra and Bhagirathi samples was suggested by allozyme data but was not found to be significant statistically for microsatellite loci. The possibility exists therefore, that mrigal may be an introduced stock in the Mahanadi river. The earliest descriptions of the mrigal distribution (Day, 1889) mentioned that IMC's were present in rivers and tanks of the Deccan plateau, a likely reference to the areas that include the Mahanadi river system. Indian major carps including mrigal occur naturally today in the Mahanadi river (Reddy, 1999). Limited differentiation and a lack of any private alleles indicate that the Mahanadi river does not support allopatric *C. mrigala* subpopulations that could have fragmented from the original distribution of the species as the Mahanadi river flowed southward through the Eastern Ghats during the Pleistocene. The likely scenario is that colonization of the *C. mrigala* in Mahanadi may have been from recent human translocations and the species has established subsequently as a naturalized population. The limited observed differentiation may have arisen due to drifting of allele frequencies at some loci rather than the accumulated impact of mutations in isolation.

Distribution of genetic variation evidenced from allozyme and microsatellite data indicate clearly the low differentiation among *C. mrigala* populations that include rivers of the Indo-Gangetic plains. Gene flow across river basins, after common ancestry, probably did not allow evolutionary forces to result in significant genetic differentiation. In the present scenario, *C. mrigala* in the Bhagirathi R. can be considered genetically divergent from populations in other localities, within the Ganges. Given the likelihood that the observed small differentiation may have been contributed to however, by mixing with farmed escapes, conservation status of this divergent 'stock' will need cautious consideration. For management of wild mrigal stocks, an important challenge will be to maintain high levels of genetic variation over time. Regulated water flows in the rivers will be crucial to maintain necessary large effective breeding population sizes that may be threatened due to reduction in flood plain areas that result from habitat alteration.

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References

Alves, M.J., Coehlo, M.M., 1994. Genetic variation and population subdivision of the endangered Iberian cyprinid *Chondrostoma lusitanicum*. J. Fish Biol. 44, 627–637.

Beacham, T.D., Pollard, S., Le, K.D., 2000. Microsatellite DNA population structure and stock identification of steelhead trout (*Oncorhynchus mykiss*) in the Nass and Skeena rivers in Northern British Columbia. Mar. Biotechnol. 2, 587–602.

Belkhir, K., Borsa, P., Goudet, J., Chikhi, L., Bonhomme, F., 1997. GENETIX ver. 4.02, Genetics logicielsous windows pour Ia ge' ne' Tique des populations. http://www.univ-montp2.fr/~genetix/genetix.htm.

Bhattacharya, S.K., 1973. Deltaic activity of Bhagirathi–Hooghly river system. J. Waterw., Harbors Coastal Eng. Div. 99 (1), 69–87.

Bouvet, Y., Soewardi, K., Pattee, E., 1991. The discrimination of Roach *Rutilus rutilus* (Linnaeus 1758) population in different parts of a river system: an investigation using Biochemical markers. Hydrobiologia 209, 161–167.

Bovo, D., Rugge, M., Shiao, Y.-H., 1999. Origin of spurious multiple bands in the amplification of microsatellite sequences. J. Clin. Pathol. Mol. Pathol. 52, 50–51.

Chapuis, M.P., Estoup, A., 2007. Microsatellite null alleles and estimation of population differentiation. Mol. Biol. Evol. 24, 621–631 (Software FreeNA available at http://www.montpellier.inra.fr/URLB/).

Chistiakov, D.A., Hellemans, B., Volckaert, F.A.M., 2006. Microsatellites and their genomic distribution, evolution, function and applications: a review with special reference to fish genetics. Aquaculture 255, 1–29.

Chondar, S.L., 1999. Biology of Finfish and Shellfish, 1st edn. SCSC Publishers, India. 514 pp.

Coelho, M.C., 1992. Genetic differentiation of the Iberian cyprinids *Chondrostoma polylepis* steind, 1865 and Ch-willkommi steind, 1866. Arch. Hydrobiol. 125, 487–498.

Coelho, M.M., Brito, R.M., Oanbeco, T.R., Figueiredo, D., Peris, A. M., 1995. Genetic variation and divergence of *Leuciscus pyrenaious* and *L. carolitertti* (Pisces, Cyprinidae). J. Fish Biol. 47 (Suppl. A), 243–258.

Daniel, R.J.R., 2001. Endemic fishes of the Western Ghats and the Satpura hypothesis. Curr. Sci. 81, 240–244.

Day, F., 1889. The Fauna of British India, including Ceylon and Burma. Fishes, vol. 1. Taylor and Francis, London. 548 pp.; 2, 509 pp.

DeWoody, J.A., Avise, J.C., 2000. Microsatellite variation in marine, freshwater and anadromous fishes compared with other animals. J. Fish Biol. 56, 461–473.

ECAFE., 1966. A compendium of major international rivers in the ECAFE region. Water Resources Series, vol. 29. United Nation Publication.

Eknath, A.E., Doyle, R.W., 1990. Effective population size and rate of inbreeding in aquaculture of Indian major carps. Aquaculture 85, 293–305.

Excoffier, Laval, L.G., Schneider, S., 2005. Arlequin ver. 3.0: an integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online 1, 47–50 (http://cmpg.unibe.ch/software/arlequin3/).

FAO, 2004. FAO year book of fisheries statistics. Aquaculture Production, vol. 90/2. Food and Agriculture Organization of United Nations, Rome.

Felsenstein, J., 1997. Population differentiation and evolutionary processes. In: Grant, W.S. (Ed.), Genetic Effects of Straying of Non-native Hatchery Fish into Natural Populations. U. S. Dept. Comm., NOAA Tech. Mem., NOAA Fisheries — NWFSC-30, pp. 31–43.

Ferguson, A., 1995. Molecular genetics in fisheries: current and future prospective. In: Carvalho, G.R., Pitcher, T.J. (Eds.), Molecular Genetics in Fisheries. Chapman and Hall, London, pp. 111–115.

Frankham, R., 1996. Relationship of genetic variation to population size in wildlife. Conserv. Biol. 10, 1500–1508.

Froese, R., Pauly, D., 2006. Fish Base. World Wide Web Electronic Publication. World Fish center (ICLARM). www.FishBase.Org.

Garcia de Leon, F.J., Chikhi, L., Bonhomme, F., 1997. Microsatellite polymorphism and population subdivision in natural populations of European sea bass *Dicentrarchus labrax* (Linnaeus 1758). Mol. Ecol. 6, 51–62.

Gopalakrishanan, A., Lal, K.K., Ponniah, A.G., 1997. Esterases in Indian major carps-Rohu (*Labeo rohita*) and Mrigal (*Cirrhinus mrigala*) (Teleostei, Cyprinidae). Indian J. Fish. 44, 361–368.

Goudet, J., Raymond, M., de Meeus, T., Rousset, F., 1996. Testing differentiation in diploid populations. Genetics 144, 933–1940.

Gyllensten, U., 1985. The genetic structure of fish: differences in the distribution of biochemical genetic variation between marine, anadromous and freshwater species. J. Fish Biol. 26, 691–699.

Hartl, D.L., Clark, A.G., 1997. Principles of Population Genetics, 3rd edn. Sinauer Associates, Inc, Sunderlands, Massachusetts. 542 pp.

Ishibashi, Y., Saitoh, T., Abe, S., Yoshida, M.C., 1997. Null microsatellite alleles due to nucleotide sequence variation in the grey-sided vole *Clethrionomys rufocanus*. Mol. Ecol. 5, 589–590.

Kimura, M., Crow, J.F., 1964. The number of alleles that can be maintained in a finite population. Genetics 49, 725–738.

Kimura, M., Ohta, T., 1978. Stepwise mutation model and distribution of allelic frequencies in a finite population. Proc. Natl. Acad. Sci. U. S. A. 75, 2868–2872.

Lakra, W.S., Krishna, G., 1996. Chromosome banding in fishes with reference to various techniques. Indian J. Anim. Sci. 66, 1306–1310.

Lal, B.B., 2002. The Saraswati Flows On: The Continuity of Indian Culture. Aryan Boobs International, New Delhi. 148 pp.

Lal, K.K., Chauhan, T., Mandal, A., Singh, R.K., Khulbe, L., Ponniah, A.G., Mohindra, V., 2004. Identification of microsatellite DNA markers for population structure analysis in Indian major carp, *Cirrhinus mrigala* (Hamilton–Buchanan, 1882). J. Appl. Ichthyol. 20, 87–91.

Lessios, H.A., 1992. Testing electrophoretic data for agreement with Hardy–Weinberg expectations. Mar. Biol. 112, 517–523.

Majumdar, K.C., Ravinder, K., Nasaruddin, K., 1997. DNA fingerprinting in Indian major carps and tilapia by Bkm 2(8) and M13 probes. Aquac. Res. 28, 129–138.

Neff, B.D., Gross, M.R., 2001. Microsatellite evolution in vertebrates: inference from AC dinucleotide repeats. Evolution 55, 1717–1733.

Nevo, E., 1978. Genetic variation in natural populations: patterns and theory. Theor. Popul. Biol. 13, 121–177.

Nevo, E., Beiles, A., Ben-Shlomo, R., 1984. The evolutionary significance of genetic diversity: ecological, demographic and life history correlates. In: Mani, G.S. (Ed.), Evolutionary Dynamics of Genetic Diversity. Springer–Verlag, Berlin, Germany, pp. 13–213.

Padhi, B.K., Gosh, S.K., Mandal, R.K., 1998. Characterization of MboI satellite in *Cirrhina mrigala* and *Clarias batrachus* (Pisces). Genome 41, 34–39.

Paetkau, D., Strobeck, C., 1995. The molecular basis and evolutionary history of microsatellite null alleles in bears. Mol. Ecol. 4, 510–520.

Payne, A.I., Sinha, R., Singh, H.R., Huq, S., 2004. A review of Ganges basin: its fish and fisheries. In: Welcomme, R., Petr, T. (Eds.), Proceedings of the second International Symposium on the Management of Large Rivers for Fisheries. Sustaining Livelihoods and Biodiversity in the New Millennium. FAO Regional Office for Asia and the Pacific, Bangkok, Thailand. RAP Publication 2004/16, vol. I.

Penman, D.J., 2005. Progress in Carp Genetics Research Carp Genetic Resources for Aquaculture in Asia. In: Penman, D.J., Gupta, M.V., Dey, M.M. (Eds.), Carp Genetic Resources for Aquaculture in Asia. WorldFish Center Technical Report, vol. 65. 152 pp.

Puri, V.M.K., Verma, B.C., 1998. Glaciological and geological source of Vedic Saraswati in the Himalayas. Itihas Darpan 2, 7–36.

Raymond, M., Rousset, F., 1995a. GENEPOP (ver. 1.2): a population genetics software for exact test and ecumenicism ver. 3.4. J. Heredity 86, 248–249 (HYPERLINK http://wbiomed.curtin.edu.au/genepop/).

Raymond, M., Rousset, F., 1995b. An exact test for population differentiation. Evolution 48, 1280–1283.

Reddy, P.V.G.K., 1999. Genetic resources of Indian major carps. FAO Fisheries Technical Paper, vol. 387. FAO, Rome. 76 pp.

Ruzzante, D.E., 1998. A comparison of several measures of genetic distance and population structure with microsatellite data: bias and sampling variance. Can. J. Fish. Aquat. Sci. 55, 1–14.

Ruzzante, D.E., Taggart, C., Cook, D., Goddard, S., 1996. Genetic differentiation between inshore and offshore Atlantic cod (*Gadus morhua*) off Newfoundland: microsatellite DNA variation and antifreeze level. Can. J. Fish. Aquat. Sci. 53, 634–645.

Salini, J.P., Milton, D.A., Rahaman, M.J., Hussein, M.G., 2004. Allozyme and morphological variation throughout the geographic range of the tropical shad, hilsa *Tenualosa ilisha*. Fish. Res. 66, 53–69.

Shaklee, J.B., Allendorf, F.W., Morizon, D.C., Whitt, G.S., 1990. Gene nomenclature for protein — coding loci in fish. Trans. Am. Fish. Soc. 119, 2–15.

Silva, E.P., Russo, C.A.M., 2000. Techniques and statistical data analysis in molecular population genetics. Hydrobiologia 420, 119–135.

Singh, R.K., Chauhan, T., Mohindra, V., Kapoor, D., Punia, P., Lal, K.K., 2004. Identification of allozyme markers for population structure analysis in *Cirrhinus mrigala* (Hamilton–Buchanan, 1882). Indian J. Fish. 51, 117–122.

Slatkin, M., 1995. A measure of population subdivision based on microsatellite allele frequencies. Genetics 139, 457–462.

Wang, D., Shi, J., Carlson, S.R., Cregan, P.B., Ward, R.W., Diers, B.W., 2003. A low-cost, high-throughput polyacrylamide gel electrophoresis system for genotyping with microsatellite DNA markers. Crop Sci. 43, 1828–1832.

Ward, R.D., Woodwark, M., Skinbinski, D.O.F., 1994. A comparison of genetic diversity levels in marine, freshwater and anadromous fishes. J. Fish Biol. 44, 213–232.

Ward, R.D., Appleyard, S.A., Daley, R.K., Reilly, A., 2001. Population structure of pink ling (*Genypterus blacodes*) from south-eastern Australian waters, inferred from allozyme and microsatellite analyses. Mar. Freshw. Res. 52, 965–973.

Weir, B.S., Cockerham, C., 1984. Estimating *F*-statistics for the analysis of population structure. Evolution 38, 1358–1370.

Whitmore, D.H., 1990. Electrophoretic and Isoelectric Focusing Techniques in Fisheries Management. CRC Press, Inc., Florida, pp. 23–80.

Wright, S., 1951. The genetical structure of populations. Ann. Eugen. 15, 323–354.

Wright, S., 1978. Evolution and the Genetics of Population, Variability within and among Natural Population. The University of Chicago Press, Chicago.

Zheng, G., Zheng, Y., Zhu, X., Luo, J., Xia, S., 1999. The genetic markers of *Cirrhina molitorella*, *C. mrigola* and *Labeo rohita* from RAPD. J. Shanghai Fish. Univ. 8, 215–220.