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Population genetic structure and phylogeography of cyprinid fish, *Labeo dero* (Hamilton, 1822) inferred from allozyme and microsatellite DNA marker analysis

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Abstract We examined population structure of *Labeo dero* (Hamilton, 1822) from different riverine locations in India using 10 polymorphic allozyme and eight microsatellite loci. For analysis, 591 different tissue samples were obtained from commercial catches covering a wide geographic range. Allozyme variability (An = 1.28–1.43, Ho = 0.029–0.071) was much lower than for microsatellites (An = 4.625–6.125, Ho = 0.538–0.633). Existence of rare alleles was found at three allozyme (*MDH-2*, GPI** and *PGDH**) and at two microsatellite loci (*R-3** and *MFW-15**). Deviation from Hardy–Weinberg equilibrium ($P < 0.05$, after the critical probability levels were adjusted for sequential Bonferroni adjustment) could be detected at three loci (*EST-1*, -2** and *XDH**) whereas, after correction for null alleles, two microsatellite loci (*MFW-1*,-15**) deviated from HWE in the river Yamuna. Fst for all the samples combined over all allozyme loci was found to be 0.059 suggesting that 5.9% of the total variation was due to genetic differentiation while microsatellite analysis yielded 0.019 which was concordant to mean Rst (0.02). Hierarchical partition of genetic diversity (AMOVA) showed that greater variability (approx. 95%) was due to within population component than between geographical regions. Based on distribution of genetic differentiation detected by both markers, at least five different genetic stocks of *L. dero* across its natural distribution could be identified. These results are useful for the evaluation and conservation of *L. dero* in natural water bodies.

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Introduction

Labeo dero or *Bangana dero* (Hamilton, 1822) (Subfamily: Cyprininae, Family: Cyprinidae) is one of the important Indian minor carps and naturally distributed all along foot hill regions of the Himalayan range in India, Nepal, Pakistan, Myanmar and China [1]. It inhabits freshwater streams at the foothill regions. In India, it is inhabitant of Indus and Ganges river systems. In the northwestern Himalayas, it is considered to be of commercial importance and experimental netting showed the species formed 3.7% of total fish catch in the region [2]. It is also identified as a potential cultivable species and promoted for higher altitude reservoirs [3]. The percentage composition of *L. dero* increased in the reservoir till 1982 and thereafter started to decline [4]. The catches during 1995–1996 to 1997–1998 ranged between 3 and 4 tones per year that was virtually insignificant [4]. In Pong Reservoir on Beas River also, due to creation of reservoir, *L. dero* among other fishes once in abundance, is struggling for its existence [4]. While regular stocking programme has been undertaken for Indian major and minor carps, for *L. dero,* it could not be made due to absence of large scale seed production technologies [4]. In India, *L. dero* is classified as one of the vulnerable species in northeastern region [5].

Stocking of Indian minor carps in rivers and reservoirs is considered a favored means to augment production from capture fisheries. Cultivable fishes like *L. dero* 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 [6]. 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. Available literature on biology and other studies on *L. dero* have been reviewed by Chonder [1]. It is a diploid ($2n = 50$) coldwater fish and the genetic research on the species is limited, restricted to karyotyping only [7]. To plan the appropriate strategy, necessary documentation of polymorphic markers and pattern of genetic variation across its natural distribution range, needs to be explored. Identification of polymorphic markers with consistently scorable alleles must be known before pattern and distribution of genetic variation in target species can be studied [6].

From genetic perspectives, the aim of natural fishery management should be to conserve intra-specific genetic diversity. Genetic variation is an important feature of population for both short term fitness of individuals and the long term survival of the population, through allowing adaptation to changing environmental conditions [6]. The genetic diversity can provide information that can used to provide scientific basis for evolving measures of conservation and management of natural population. Information deduced from molecular markers can provide insight into genetic structure and also early warnings of genetic bottleneck that population may be facing. Until now, there is no recorded information available about genetic variation of natural population in *Labeo dero*.

There are wide array of molecular markers that include analysis of protein and DNA. A combination of molecular assay of DNA and protein markers may provide the best approach to understand the genetic diversity of related population [8]. Microsatellite and allozyme markers have been extensively used for direct assessment of pattern of genetic variation as these are loci with co-dominant alleles inherited in Mandelian fashion. Due to their co-dominant nature, these two markers provide robustness in statistical analysis especially to draw inference if the population follows Hardy–Weinberg equilibrium or not. Allozyme and microsatellite markers have been used independently or in combination to determine genetic variation and draw inference on population structure in fishes and shellfishes [9] and to unearth population level evolution pattern for variety of vertebrates [10–12].

Polymorphic allozyme and microsatellite markers [13, 14] in *L. dero* have been developed and these markers appear to be promising for assessing genetic differentiation in natural *Labeo dero* populations across its distribution range. The present study assessed the natural population structure of *L. dero* on a macro-geographical scale.

Materials and methods

Fish samples

A total of 591 samples of *L. dero* from commercial catches were collected from ten different riverine sites (Table 1). Sampling sites were selected to document genetic variation across a wide geographical distribution range (32°66′N, 75°40′E to 18°57′N, 79°06′E) (Fig. 1). Beas and Satluj are part of Indus river system. Ganga, Yamuna, Kosi, Gerua, Tons and Brahmaputra are distant but associated rivers of the Ganges river system, while Mahanadi forms different river systems [15]. Tissue samples (liver and muscle) were excised at the collection sites and immediately immersed in liquid nitrogen (−196°C). The samples in frozen state were transported to laboratory and were stored at −80°C until analysed. The blood samples were collected through caudal puncture and fixed in 95% ethanol (1:5) at 4°C.

To analyze allozyme markers, frozen liver tissues (approx 100 mg) were homogenized in 500 mg ml^{-1} extraction buffer (0.17 M Sucrose, 0.2 M EDTA, 0.2 M Tris–HCl, pH 7.0) and centrifuged for 1 h at 10,000 rpm at 4°C. The supernatant thus obtained, was re-centrifuged for 20 min. Allelic variation was investigated using 8% polyacrylamide gel electrophoresis. Electrophoresis was carried out at constant voltage 150 V at 4°C. The enzyme activity zones were stained with histochemical stains [16]. The optimized parameters followed for the allozyme analysis of 569 individuals were based on our previous work [13].

Genomic DNA was extracted from blood via a protocol modified from Ruzzante et al. [17], using proteinase K, and phenol:chloroform. For microsatellite analysis, five polymorphic loci *MFW-1** (FJ491395), 15*(FJ491398), 17*(FJ491399), 24*(FJ491396), 26* (FJ491397) were available from our earlier work [14]. To obtain more polymorphic loci, 31 primers developed for cyprinid fishes, *Barbodes gonionotus* (AJ291680-84), *Labeo rohita* (AJ507518-22; AJ507524), *Cyprinus carpio* (AY169249-50; AB043469), *Campostoma anomalum* (AF277575,77,78,80,82-84,88-89; AF277587) and *Pimephales promelas* (AY254350-54,56) were cross primed in *L. dero*. In this study, PCR amplification of each DNA sample was performed in a 25 µl reaction mixture, which contains 25–50 ng of DNA, 1× buffer (10 mM Tris HCl pH 9.0, 50 mM KCl, 0.01 gelatin), 0.5 mM $MgCl_2$, 0.2 mM of each dNTPs, 0.5 unit of *Taq* DNA polymerase and 5 pmol of each primer. The amplifications were carried in a thermal cycler PTC-200 MJ Research, with conditions: 94°C for 5 min followed by 25 cycles of 30 s at 94°C, at annealing temperature for 30 s, 72°C for 1 min and one cycle at 72°C for 4 min. Amplicons were resolved on 10% non-denaturing PAGE with 1× TBE buffer for 5 h at constant voltage of 150 V at 4–6°C. The gels were silver stained (silver staining kit, Pharmacia Biotech, USA) and known DNA size

Table 1 Sample size, location, year of collection of *Labeo dero* from different rivers in India

S. no.	River system	River/reservoir	Location site	Location (lat. & log.)	Year	Sample size (*N*)
1	Indus	Beas	Pathankot, Punjab	32°66′N	April, 2003	75
				75°40′E		
		Satluj	Nangal, Punjab	31°23′N	September, 2000	37
				75°30′E	April, 2003	36
2	Ganges	Ganga	Ajetpur, Uttarachal	29°58′N	March 2001	79
				78°10′E	October, 2003	21
		Kosi	Ramnagar, U.P.	29°24′N	April, 2003	33
				79°07′E	November, 2003	31
		Gerua	Katrnia Ghat, U.P.	32°19′N	November, 2003	74
				75°30′E		
		Yamuna	Tajewala, Haryana	30°09′N	September, 2000	8
				77°21′E	March–April, 2001	86
		Tons	Rewa, M.P.	24°31′N	April, 2004	20
				81°17′E		
3	Brahmaputra	Jiabharali	Bhaluk Pong, Arunachal	27°28′N	March, 2003	56
				94°15′E		
4	Mahanadi	Mahanadi	Sonepur, Orissa	20°50′N	May, 2004	33
				83°56′E		
5	Godavari	Godavari	Warrangal, A.P.	18°57′N	October, 2002	02
				79°06′E		
Total						591

marker *(MspI* cut pBR 322 DNA) was run in every gel. The size of the amplified products was determined with ID Elite (Amersham Biosciences). In this investigation, native PAGE was used as, the 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 analyzing large sample sizes [18]. Moreover, Bovo et al. [19] demonstrated that non denaturing electrophoresis is not responsible for spurious or multiple bands in microsatellite analysis.

Statistical analysis

Individual fish genotypes at each allozyme and microsatellite locus were determined. The datasets were then analyzed for homogeneity between sample collections at different times and neighboring localities within each river. Data sets within each river or neighboring tributaries that were not heterogeneous ($P > 0.05$) were later combined to further estimate the genetic variation and differentiation in *L. dero*. A locus was considered polymorphic, if the frequency of the most common allele was less than or equal to 0.99 [20]. Software Genetix 4.05.2 [21] was used to obtain allele frequencies, mean number of alleles per locus, heterozygosity values (expected H_{exp} and observed H_{obs}) at individual allozyme and microsatellite loci. In case of microsatellite loci, null alleles were determined using FreeNA software [22].

Tests for conformity to Hardy–Weinberg expectations (probability test) and linkage disequilibrium were undertaken in Genepop ver. 3.4 software [23]. Genetic heterogeneity of all population and pair-wise localities were determined using an exact test (*G* based test) that assumes random samples of genotypes (Genepop ver. 3.4, Genotype differentiation test, 22). This test is performed on genotype tables and possible non-independence of alleles within genotypes does not affect test validity [24, 25]. The null hypothesis tested was that the genotype distribution was identical across all populations.

Genetix ver. 4.05 software was used to estimate *F*-statistics [26] computed as estimators θ, F and f of Weir and Cockerham [27]. Probability of θ significantly deviating from zero was calculated using 1000 bootstraps. Allelic homogeneity for both the markers was calculated using software Genepop. The hierarchical analysis was carried out using analysis of molecular variance (AMOVA) in the Arlequin 2000 package [28]. AMOVA estimations were done at three levels of the population structure: within population, among subpopulations within river basins and among river basins. Under a SMM model, estimates of Rst was made for microsatellite loci using the Genepop. To correct for multiple simultaneous comparisons, sequential

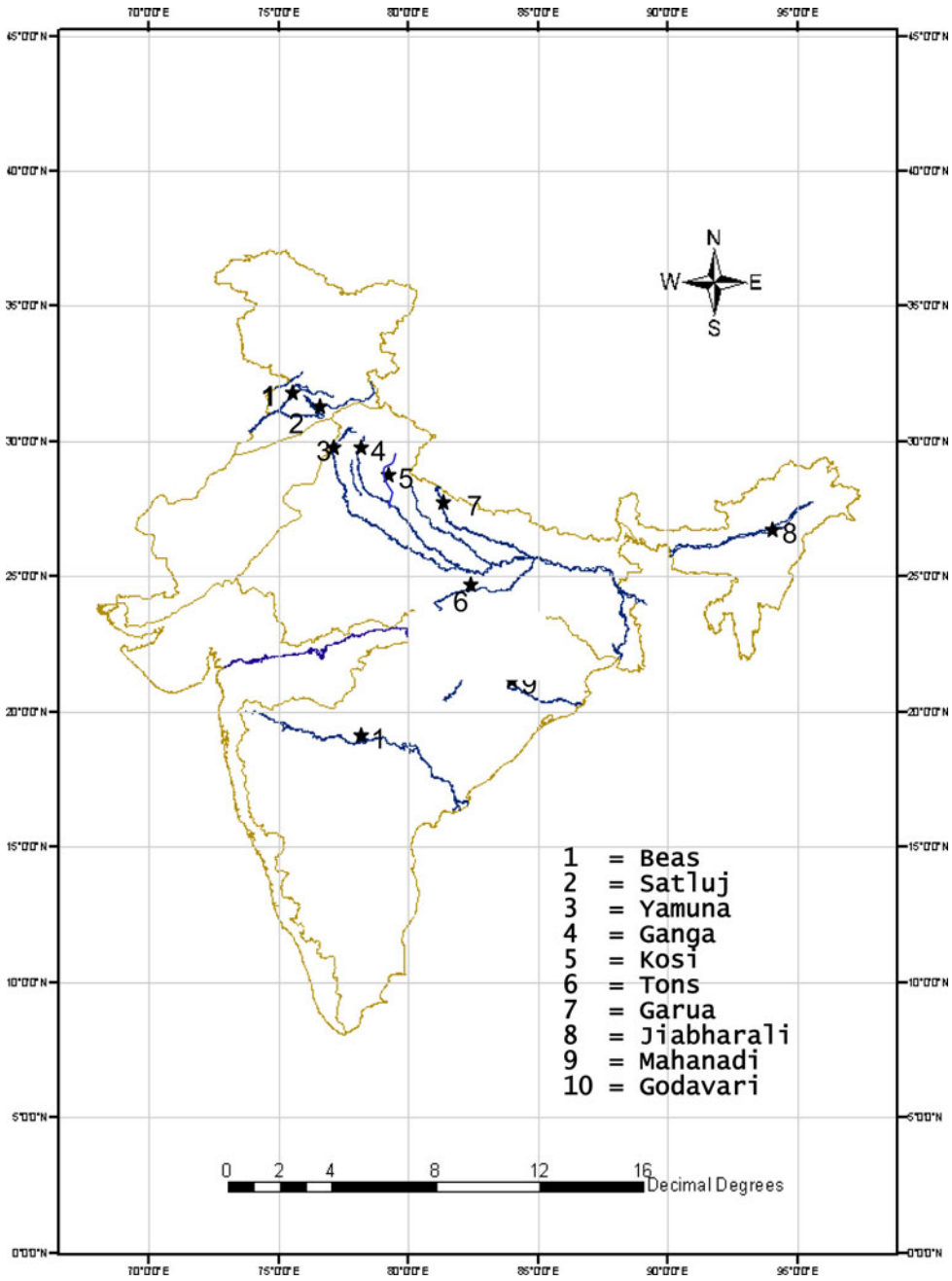

Fig. 1 Sample collection sites (*filled star*) of *Labeo dero* during present study

Bonferroni corrections were applied using a global significance level of 0.05 [29].

To detect whether the *L. dero* populations have experienced a recent reduction in the effective population size, software Bottleneck [30] was used. Infinite allele model (IAM) was used for allozyme loci, whereas for microsatellite loci, combined models of SMM and TPM with a contribution of two forms of mutation with 70% SMM and 30% IAM were used [30]. These models have no bias towards an expansion or a contraction, and thus the microsatellites are expected to grow or contract unconstrained over time [31, 32].

Results

Allozyme analysis

A total of 28 protein-coding loci (from 18 enzyme systems) were detected and provided interpretable results for population analysis. Among 28 presumptive loci, eight loci (*EST-1*, EST-2*, GPDH*, PGDH*, XDH*, GPI*, PGM-2** and *MDH-2**) were polymorphic. Three loci *(MDH-2*, GPI** and *PGDH**) were found to possess rare alleles in low frequency (equal or less than 0.01) in Brahmaputra, Ganga (1st, & 4th allele) and Tons (3rd allele), respectively (Table 2).

Table 2 Allele frequencies of eight polymorphic allozyme loci in *Labeo dero* from nine different locations

Population Locus	Beas 1	Satluj 2	Ganga 3	Yamuna 4	Kosi 5	Tons 6	Gerua 7	Brahmaputra 8	Mahanadi 9
*EST-1**									
(N)	73	70	75	91	61	18	70	53	28
92	0.0548	0.0714	0.0867	0.0549	0.1639	0.0278	0.1643	0.0849	0.2321
100	0.8904	0.8643	0.6933	0.7088	0.7049	0.5278	0.7643	0.6415	0.5536
106	0.0548	0.0643	0.2200	0.2363	0.1311	0.4444	0.0714	0.2736	0.2143
*EST-2**									
(N)	72	69	75	91	61	18	70	53	29
96	0.0000	0.0000	0.0000	0.0220	0.0000	0.3056	0.0000	0.0000	0.0345
100	0.5347	0.4565	0.3800	0.4341	0.5492	0.4444	0.6643	0.3774	0.2759
108	0.2778	0.4638	0.3867	0.4011	0.2869	0.0833	0.2071	0.4340	0.5862
113	0.1875	0.0797	0.2333	0.1429	0.1557	0.1389	0.1286	0.1604	0.1034
115	0.0000	0.0000	0.0000	0.0000	0.0082	0.0278	0.0000	0.0283	0.0000
*GPDH**									
(N)	73	68	68	80	57	18	70	52	19
84	0.0068	0.0074	0.0147	0.0188	0.0263	0.1389	0.0000	0.0288	0.0526
100	0.9932	0.9926	0.9191	0.9688	0.9474	0.8611	0.9786	0.9423	0.8684
113	0.0000	0.0000	0.0662	0.0125	0.0263	0.0000	0.0214	0.0288	0.0789
*GPI**									
(N)	73	71	75	92	62	18	70	54	29
71	0.0000	0.0000	**0.0067**	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
85	0.0000	0.0000	0.0000	0.0054	0.0161	0.0000	0.0000	0.0000	0.0000
100	1.0000	1.0000	0.9867	0.9946	0.9839	1.0000	1.0000	1.0000	1.0000
115	0.0000	0.0000	**0.0067**	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
*XDH**									
(N)	73	64	71	85	57	18	70	47	27
97	0.0068	0.0000	0.0000	0.0000	0.0000	0.2500	0.0571	0.0319	0.0556
100	0.6575	0.7578	0.6901	0.5176	0.5965	0.5278	0.4143	0.2766	0.3889
108	0.3356	0.2422	0.3099	0.4824	0.4035	0.2222	0.5286	0.6915	0.5556
*PGDH**									
(N)	73	63	71	86	56	18	70	54	24
100	0.8493	0.7937	0.7887	0.6919	0.7589	0.3056	0.8857	0.9907	0.7708
113	0.1507	0.2063	0.2113	0.3081	0.2411	0.5833	0.1143	0.0093	0.2292
126	0.0000	0.0000	0.0000	0.0000	0.0000	**0.1111**	0.0000	0.0000	0.0000
*PGM-2**									
(N)	73	71	75	92	60	18	70	54	29
100	1.0000	0.9859	0.9933	1.0000	1.0000	0.9167	0.9929	1.0000	0.9828
113	0.0000	0.0141	0.0067	0.0000	0.0000	0.0833	0.0071	0.0000	0.0172
*MDH-2**									
(N)	73	71	75	92	62	18	70	54	29
100	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9815	1.0000
115	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	**0.0185**	0.0000

Private alleles are given in bold

N No. of samples analysed

Allele frequencies at eight allozyme loci are given in Table 2 and summary statistics for parameters of genetic variation at each allozyme loci are given in Table 3. The mean number of alleles per locus ranged from 1.28 (Beas and Satluj) to 1.43 (Tons), while the observed heterozygosity varied between 0.029 (Beas) and 0.071 (Tons).

Table 3 Parameters of genetic variation for eight polymorphic allozyme loci in *Labeo dero* from nine different locations

S. no.	Population Locus	Beas 1	Satluj 2	Ganga 3	Yamuna 4	Kosi 5	Tons 6	Gerua 7	Brahmaputra 8	Mahanadi 9
1.	*EST-1**									
	Hobs.	0.0685	0.0714	0.1733	0.1758	0.1803	0.2778	0.2286	0.1698	0.4643
	Hexp.	0.2012	0.2438	0.4634	0.4388	0.4590	0.5231	0.3838	0.5064	0.5938
	P_{HW}	0.0000**	0.0000**	0.0000**	0.0000**	0.0000**	0.0307*	0.0002**	0.0000**	0.0248*
	P_{score}	0.0000**	0.0000**	0.0000**	0.0000**	0.0000**	0.0307*	0.0005**	0.0000**	0.0215*
	F_{is}	+0.663	+0.711	+0.630	+0.603	+0.612	+0.491	+0.410	+0.670	+0.235
2.	*EST-2**									
	Hobs.	0.3333	0.3768	0.4400	0.3956	0.2787	0.5556	0.4286	0.4151	0.3793
	Hexp.	0.6018	0.5702	0.6516	0.6298	0.5918	0.6821	0.4993	0.6428	0.5684
	P_{HW}	0.0000**	0.0014*	0.0004**	0.0000**	0.0000**	0.0192*	0.1494	0.0046*	0.0038*
	P_{score}	0.0000**	0.0221*	0.0001**	0.0015**	0.0000**	0.0368*	0.0741	0.0093*	0.0677
	F_{is}	+0.452	+0.346	+0.331	+0.377	+0.535	+0.213	+0.149	+0.362	+0.348
3.	*GPDH**									
	Hobs.	0.0137	0.0147	0.1618	0.0625	0.1053	0.1667	0.0429	0.0769	0.2632
	Hexp.	0.0136	0.0146	0.1506	0.0610	0.1011	0.2392	0.0419	0.1104	0.2368
	P_{HW}	–	–	1.0000	1.0000	1.0000	0.2727	1.0000	0.0591	1.0000
	P_{score}	–	–	0.6448	0.9377	0.8708	0.2727	0.9784	0.0574	0.7413
	F_{is}	–	–	−0.067	−0.018	−0.032	+0.329	−0.015	+0.312	−0.084
4.	*GPI**									
	Hobs.	0.0000	0.0000	0.0267	0.0109	0.0323	0.0000	0.0000	0.0000	0.0000
	Hexp.	0.0000	0.0000	0.0264	0.0108	0.0317	0.0000	0.0000	0.0000	0.0000
	P_{HW}	–	–	1	–	1	–	–	–	–
	P_{score}	–	–	0.9933	–	0.9919	–	–	–	–
	F_{is}	–	–	−0.003	–	−0.008	–	–	–	–
5.	*XDH**									
	Hobs.	0.1918	0.2344	0.2817	0.2824	0.2456	0.5556	0.2714	0.2128	0.3333
	Hexp.	0.4550	0.3671	0.4277	0.4994	0.4814	0.6096	0.5457	0.4443	0.5370
	P_{HW}	0.0000**	0.0056*	0.0051*	0.0001**	0.0003**	0.6773	0.0000**	0.0000**	0.0071*
	P_{score}	0.0000**	0.0056*	0.0042*	0.0000**	0.0002**	0.2311	0.0000**	0.0253*	0.0629
	F_{is}	+0.583	+0.368	+0.348	+0.439	+0.496	+0.117	+0.508	+0.529	+0.395
6.	*PGDH**									
	Hobs.	0.1918	0.3175	0.2817	0.3837	0.3393	0.2778	0.2000	0.0185	0.1250
	Hexp.	0.2560	0.3275	0.3333	0.4264	0.3659	0.5540	0.2024	0.0183	0.3533
	P_{HW}	0.0468*	0.7123	0.2774	0.4461	0.7128	0.0111*	1.0000	–	0.0044*
	P_{score}	0.0468	0.5116	0.1539	0.2302	0.3894	0.0048*	0.6195	–	0.0044*
	F_{is}	+0.257	+0.039	+0.162	+0.106	+0.082	+0.520	+0.019	–	+0.658
7.	*PGM-2**									
	Hobs.	0.0000	0.0282	0.0133	0.0000	0.0000	0.1667	0.0143	0.0000	0.0345
	Hexp.	0.0000	0.0278	0.0132	0.0000	0.0000	0.1528	0.0142	0.0000	0.0339
	P_{HW}	–	1.0000	–	–	–	1.0000	–	–	–
	P_{score}	–	0.9929	–	–	–	0.9143	–	–	–
	F_{is}	–	−0.007	–	–	–	−0.063	–	–	–
8.	*MDH-2**									
	Hobs.	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0370	0.0000
	Hexp.	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0364	0.0000
	P_{HW}	–	–	–	–	–	–	–	1.0000	–
	P_{score}	–	–	–	–	–	–	–	0.9907	–
	F_{is}	–	–	–	–	–	–	–	−0.010	–

Table 3 Parameters of genetic variation for eight polymorphic allozyme loci in *Labeo dero* from nine different locations

S. no.	Population Locus	Beas 1	Satluj 2	Ganga 3	Yamuna 4	Kosi 5	Tons 6	Gerua 7	Brahmaputra 8	Mahanadi 9
	Overall loci									
	Hobs.	0.0285	0.0372	0.0492	0.0468	0.0422	0.0714	0.0423	0.0332	0.0571
	Hexp.	0.0546	0.0554	0.0738	0.0738	0.0725	0.986	0.0603	0.0628	0.0830
	P(0.95)	0.1429	0.1429	0.1786	0.1429	0.1786	0.2143	0.1429	0.1429	0.1786
	P(0.99)	0.1429	0.1786	0.2143	0.1786	0.2143	0.2143	0.1786	0.1786	0.2143
	A_n	1.2857	1.2857	1.3929	1.3571	1.3571	1.4286	1.3214	1.3929	1.3929

Hobs. observed heterozygosity, *Hexp.* expected heterozygosity, F_{is} inbreeding coefficient, P_{HW} probability value of significant deviation from Hardy–Weinberg equilibrium, P_{score} probability value of significant heterozygosity deficiency, *P*(0.95) polymorphism at 0.95 criteria, *P*(0.99) polymorphism at 0.99 criteria, A_n mean number of alleles per locus

* Significant ($P < 0.05$)

** Significant after Bonferroni adjustment

Deviation from Hardy–Weinberg equilibrium ($P < 0.05$, after the critical probability level were adjusted for sequential Bonferroni adjustment) could be detected at three loci (*EST-1*, EST-2** and *XDH**). No test for linkage disequilibrium was statistically significant for any pair of allozyme loci within each of the sample sites and when all the samples were considered together. Existence of genetic bottleneck at allozyme loci was analyzed under Infinite allele model (IAM) of mutation (Table 8). The estimates provided the evidence of occurrence of bottleneck in the samples from Tons exhibiting significant probabilities. Mode-shift in allele frequency distribution was not found at any population using allozymes.

Microsatellite analysis

The cross priming of 31 primers from cyprinid fish species successfully amplified three loci; *R3** (FJ491403), *R12** (FJ491402), *Ca12** (FJ491401) that were polymorphic for use in genotyping with five primers already available from our earlier work. A total of eight microsatellite loci (*MFW-1*, 15*, 17*, 24*, 26*, Ca-12*, R-3** and *-12**) were used to genotype individuals ($n = 591$) of *L. dero*. Individual fish genotype data from the multiple collections within the same river (collected at different time intervals) was tested for homogeneity. No significant heterogeneity in genotype proportions was detected between such sample sets within the rivers Satluj, Ganga, Kosi, Tons and Yamuna. After pooling, nine genotype data sets were available to analyze genetic variation and differentiation among *L. dero* populations. Distribution of allele frequencies at polymorphic microsatellite loci in *L. dero* samples from the nine localities is presented in Table 4. No population specific alleles were observed at any microsatellite locus. Rare alleles were found in samples from Gerua and Ganga rivers at loci *R-3** and *MFW-15**. Evidences of null alleles were found at all microsatellite loci (in all populations) with a lowest estimated allele frequency in river Mahanadi and highest in river Brahmaputra.

Summary statistics for parameters of genetic variation at individual loci and across all loci (Table 5) and values (Table 6) for inbreeding coefficient (Fis) were computed. The mean number of alleles per locus ranged from 4.6250 (Beas) to 6.1250 (Gerua), after correction for null alleles. The observed heterozygosity values ranged from 0.5380 (Yamuna) to 0.6338 (Mahanadi). No test for linkage disequilibrium was statistically significant for any pair of microsatellite loci within each of the sample sites and when all samples were considered together. For microsatellite loci, before correction for null alleles, test for conformity to Hardy–Weinberg expectations (after the critical probability level were adjusted for sequential Bonferroni adjustment), indicated that allele frequencies deviated significantly (Table 7) at seven loci except *Ca12**, However, after correction for null alleles, two loci (*MFW-1*,-15**), were in found to be significantly deviating from Hardy–Weinberg equilibrium in samples from river Yamuna. Score test revealed one more locus (*R12**) that expressed deviation from HWE (in Ganga samples) with marginal excess of heterozygotes (−0.003).

The genetic bottleneck studies based on microsatellite loci as analyzed under Two Phase model (Table 8). After correction for null alleles, significant probabilities under TPM (for excess of heterozygotes) was observed, in samples from Satluj, (Wilcoxon $P = 0.03711$), Kosi (Wilcoxon $P = 0.00977$), Tons (Wilcoxon $P = 0.03711$) and Gerua (Wilcoxon $P = 0.01953$) localities. Another approach, which involves tracing of mode shift of allele frequencies distribution, was also used to determine the bottleneck. Mode-shift was observed in the samples of river Mahanadi only (Fig. 2).

Table 4 Allele frequencies of eight polymorphic microsatellite loci across nine collection sites in *Labeo dero*

Population Locus	Beas 1	Satluj 2	Ganga 3	Yamuna 4	Kosi 5	Tons 6	Gerua 7	Brahmaputra 8	Mahanadi 9
*MFW-1**									
(N)	73	69	91	87	63	19	73	55	30
169	0.4110	0.3333	0.3462	0.3161	0.4048	0.1579	0.3493	0.3364	0.1333
171	0.1644	0.1304	0.0440	0.0805	0.0794	0.0263	0.1301	0.1000	0.1333
173	0.2671	0.3913	0.4560	0.3736	0.3492	0.3947	0.3630	0.3545	0.4500
177	0.0890	0.0725	0.0769	0.0805	0.0794	0.2632	0.0685	0.0636	0.1500
181	0.0411	0.0652	0.0549	0.0862	0.0397	0.1053	0.0342	0.1000	0.1167
189	0.0274	0.0072	0.0220	0.0632	0.0476	0.0526	0.0548	0.0455	0.0167
*MFW-15**									
(N)	75	69	93	91	65	19	72	54	30
136	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	**0.0069**	0.0000	0.0000
148	0.1200	0.0580	0.0376	0.1319	0.1154	0.1316	0.0833	0.1481	0.0833
150	0.7267	0.7464	0.5968	0.5659	0.6154	0.8158	0.6181	0.5556	0.6167
154	0.0133	0.0072	0.0161	0.0000	0.1154	0.0000	0.0208	0.0741	0.1167
158	0.1200	0.1377	0.1989	0.1923	0.1308	0.0263	0.2014	0.1667	0.1333
160	0.0200	0.0507	0.1344	0.1099	0.0231	0.0263	0.0556	0.0556	0.0500
166	0.0000	0.0000	**0.0161**	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
170	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	**0.0069**	0.0000	0.0000
198	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	**0.0069**	0.0000	0.0000
*MFW-17**									
(N)	75	69	94	90	64	19	69	55	29
186	0.0000	0.0000	0.0106	0.0167	0.0078	0.0000	0.0000	0.0000	0.0000
194	0.0000	0.0000	0.0160	0.0056	0.0156	0.0526	0.0072	0.0000	0.0000
198	0.0267	0.0507	0.1117	0.0889	0.1016	0.0789	0.0652	0.0727	0.1552
204	0.6000	0.6812	0.5319	0.6222	0.5859	0.3684	0.5435	0.4091	0.6034
208	0.2733	0.1522	0.1755	0.1444	0.1797	0.2368	0.2246	0.2273	0.1207
210	0.0000	0.0000	0.0426	0.0111	0.0234	0.0000	0.0507	0.0455	0.0517
214	0.1000	0.1159	0.1117	0.1111	0.0859	0.2632	0.1087	0.2455	0.0690
*MFW-24**									
(N)	75	69	94	89	65	19	73	55	30
159	0.0000	0.0000	0.0053	0.0000	0.0154	0.0263	0.0068	0.0000	0.1000
161	0.9867	0.9855	0.9574	0.9888	0.9692	0.8947	0.9521	0.9000	0.7833
165	0.0133	0.0145	0.0372	0.0112	0.0154	0.0789	0.0411	0.1000	0.1167
*MFW-26**									
(N)	75	69	94	91	65	19	73	55	30
106	0.0600	0.0362	0.0160	0.0440	0.1231	0.0789	0.0068	0.1091	0.4333
110	0.8933	0.9275	0.9255	0.8736	0.8154	0.8947	0.9452	0.8091	0.5167
114	0.0467	0.0362	0.0585	0.0769	0.0538	0.0263	0.0479	0.0636	0.0500
118	0.0000	0.0000	0.0000	0.0055	0.0077	0.0000	0.0000	0.0182	0.0000
*Ca-12**									
(N)	75	66	93	90	65	19	73	55	30
152	0.0000	0.0076	0.0000	0.0000	0.0000	0.0000	0.0000	0.0182	0.0000
164	0.0000	0.0076	0.0054	0.0056	0.0308	0.0000	0.0137	0.0273	0.0333
176	0.0400	0.1136	0.1720	0.1278	0.0769	0.0263	0.1027	0.1545	0.1500
180	0.6000	0.5076	0.4301	0.4944	0.4692	0.5789	0.4452	0.4091	0.2833
184	0.3600	0.3561	0.3602	0.3500	0.3923	0.1842	0.3493	0.3818	0.4333
188	0.0000	0.0000	0.0000	0.0000	0.0231	0.1579	0.0479	0.0000	0.0833
192	0.0000	0.0076	0.0323	0.0222	0.0077	0.0526	0.0411	0.0091	0.0167

Table 4 continued

Population Locus	Beas 1	Satluj 2	Ganga 3	Yamuna 4	Kosi 5	Tons 6	Gerua 7	Brahmaputra 8	Mahanadi 9
*R-12**									
(N)	59	58	84	80	58	5	62	43	23
115	0.3729	0.3362	0.3512	0.3000	0.3362	0.8000	0.4032	0.4884	0.0435
117	0.1102	0.1293	0.1429	0.2062	0.1552	0.0000	0.1048	0.1163	0.0435
119	0.5169	0.5086	0.4762	0.4750	0.4483	0.2000	0.4355	0.3605	0.8043
124	0.0000	0.0259	0.0298	0.0063	0.0431	0.0000	0.0484	0.0349	0.1087
134	0.0000	0.0000	0.0000	0.0125	0.0172	0.0000	0.0081	0.0000	0.0000
*R-3**									
(N)	75	66	93	87	65	19	73	55	30
92	0.0000	0.0000	0.0108	0.0460	0.0077	0.1053	0.0342	0.0000	0.1167
104	0.1000	0.1212	0.1989	0.1839	0.1231	0.3421	0.1712	0.2636	0.2500
108	0.3000	0.2197	0.1452	0.0862	0.1615	0.0526	0.1918	0.1000	0.2667
110	0.5000	0.5152	0.5806	0.6149	0.6385	0.4737	0.5479	0.5818	0.2667
118	0.1000	0.1439	0.0645	0.0690	0.0692	0.0263	0.0479	0.0545	0.1000
128	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	**0.0068**	0.0000	0.0000

Private alleles are given in bold

N No. of samples analysed

Population genetic structure

Analysis of *L. dero* samples from nine riverine locations exhibited significant variation at seven allozyme and eight microsatellite loci. The combined probability over all loci (allozyme and microsatellite) was highly significant ($P > 0.0001$, Table 9) indicating that samples drawn are not part of same gene pool. Examination of genotype data at each microsatellite locus over all populations revealed that all eight loci (*MFW-1*, 15*, 17*, 24*, 26*, Ca-12*, R-3*, 12**) had significant heterogeneity ($P < 0.005$) after nominal levels of significance were adjusted (Table 9). Seven out of 28 allozyme loci (*EST-1*, EST-2*, XDH*, PGDH*, GPDH*, PGM-2** and *MDH-2**) had significant heterogeneity ($P < 0.005$). This suggested that the genetic variation detected at these microsatellite and allozyme loci is significant to detect genotypic differences and can be useful in analyzing population structure of *L. dero*.

At allozyme loci, the mean F_{st} of all the loci across all collections was 0.059, that indicated that 5.9% of the total variability between the populations is due to inter-population differences**.** The pair-wise F_{st} values ranged from 0.01496 (Beas and Satluj) to 0.24492 (Jiabharali and Tons) (Table 10). Pair-wise comparisons of samples from nine localities, indicated that 25 out of 36 possible pairs had significant genetic heterogeneity, and over all loci after the Sequential Bonferroni correction was applied to the probability level. After locus-wise examination, all these population pairs were found to display significant genotype heterogeneity at least at one locus. Within the *L. dero* populations, percent variation was found to be higher (95%) while among basins the estimate was lower (2.3%).

In *L. dero* natural population in different rivers, the mean Fst value over all collections and all microsatellite loci (Table 11) was found to be 0.019 indicating that 1.9% of the total genetic variability can be attributed to genetic differentiation. The pair-wise Fst values ranged from 0.00507 (Beas and Satluj) to 0.14001 (Mahanadi and Tons). At microsatellite loci, 20 out of 36 pair-wise comparisons were statistically significant. The mean Rst value (0.022) was comparable to average Fst estimates of microsatellite markers. AMOVA analysis revealed 95.2% of genetic variation to be present within *L. dero* populations; whereas small variation was evident among the river basins (1.96%) and among populations within the groups (2.84%).

Discussion

The present study provided insight into genetic differentiation in natural population of *L. dero*. The genetic variation detected with both polymorphic allozyme and microsatellite DNA markers was analyzed to compute parameters of genetic differentiation. For allozyme loci, genetic variability in *L. dero* (Ho = 0.029–0.071, mean no. of alleles per locus 1.28–1.43) was comparable to values described for many freshwater fish species [33, 34]. Observed heterozygosity values were also within the range reported for teleost fish species (0.05–0.07) described by Nevo [35]. As

Table 5 Heterozygosities values and mean number of alleles (A_n) for eight polymorphic microsatellite loci in *Labeo dero* from nine riverine sites

S. no.	Population Locus	Beas 1	Satluj 2	Ganga 3	Yamuna 4	Kosi 5	Tons 6	Gerua 7	Brahmaputra 8	Mahanadi 9
1.	*MFW-1**									
	Hobs.	0.3425	0.4203	0.4835	0.4023	0.5873	0.5263	0.4658	0.4727	0.4667
		0.7778	0.7681	0.6813	0.7701	0.7143	0.7368	0.7671	0.7273	0.7333
	Hexp.	0.7224	0.7092	0.6609	0.7362	0.6978	0.7355	0.7204	0.7350	0.7256
		0.7809	0.7737	0.7186	0.7976	0.7309	0.7839	0.7777	0.7916	0.7789
2.	*MFW-15**									
	Hobs.	0.3200	0.2319	0.4409	0.4176	0.4308	0.1579	0.4722	0.4815	0.4667
		0.5467	0.5652	0.6452	0.7033	0.6308	0.4737	0.6250	0.6667	0.6333
	Hexp.	0.4426	0.4180	0.5843	0.6133	0.5770	0.3158	0.5668	0.6331	0.5789
		0.5394	0.5494	0.6518	0.6822	0.6475	0.4626	0.6190	0.6860	0.6372
3.	*MFW-17**									
	Hobs.	0.2400	0.3768	0.5213	0.4778	0.4688	0.2632	0.5797	0.5818	0.3793
		0.6667	0.5652	0.6809	0.6000	0.6563	0.7895	0.6667	0.7091	0.6552
	Hexp.	0.5546	0.4968	0.6591	0.5713	0.6058	0.7299	0.6355	0.7134	0.5898
		0.6737	0.5707	0.7054	0.6197	0.6665	0.7825	0.6624	0.7474	0.6760
4.	*MFW-24**									
	Hobs.	0.0000	0.0000	0.0426	0.0225	0.0308	0.2105	0.0685	0.0545	0.3000
		0.1600	0.1594	0.2128	0.0225	0.1846	0.2105	0.1781	0.3636	0.4333
	Hexp.	0.0263	0.0286	0.0819	0.0222	0.0601	0.1925	0.0919	0.1800	0.3628
		0.1594	0.1599	0.2124	0.0222	0.1838	0.1925	0.1784	0.3737	0.4378
5.	*MFW-26**									
	Hobs.	0.0800	0.0870	0.0638	0.2198	0.2154	0.1053	0.0548	0.1636	0.3333
		0.3867	0.2754	0.3085	0.2308	0.4154	0.3684	0.2466	0.4909	0.5667
	Hexp.	0.1962	0.1371	0.1397	0.2289	0.3170	0.1925	0.1042	0.3291	0.5428
		0.3757	0.2705	0.3034	0.2384	0.4317	0.3560	0.2448	0.4868	0.6344
6.	*Ca-12**									
	Hobs.	0.5333	0.4848	0.6452	0.5111	0.6615	0.5263	0.7534	0.6182	0.5000
		0.5867	0.6364	0.6452	0.6556	0.7077	0.6316	0.8082	0.7091	0.7667
	Hexp.	0.5088	0.6025	0.6546	0.6162	0.6185	0.6025	0.6650	0.6618	0.7011
		0.5292	0.6561	0.6546	0.6608	0.6314	0.6385	0.6757	0.6881	0.7511
7.	*R-12**									
	Hobs.	0.2373	0.2759	0.2381	0.2750	0.2931	0.0000	0.3548	0.2093	0.1304
		0.6949	0.7069	0.7349	0.7375	0.7586	0.6000	0.7258	0.7209	0.5217
	Hexp.	0.5816	0.6109	0.6286	0.6416	0.6598	0.3200	0.6344	0.6168	0.3374
		0.6933	0.6999	0.7147	0.7304	0.7375	0.5400	0.7133	0.7185	0.5009
8.	*R-3**									
	Hobs.	0.7067	0.4848	0.6129	0.4483	0.6308	0.7368	0.7671	0.4364	0.7333
		0.7067	0.6327	0.6067	0.5362	0.6190	0.6667	0.7500	0.5333	0.7600
	Hexp.	0.6400	0.6509	0.5979	0.5737	0.5463	0.6440	0.6301	0.5790	0.7717
		0.6400	0.6420	0.5918	0.5428	0.5305	0.5889	0.6118	0.5840	0.7200
9.	Overall loci									
	Hobs.	0.3075	0.2952	0.3810	0.3468	0.4148	0.3158	0.4395	0.3773	0.4137
		0.5658	0.5387	0.5387	0.5320	0.5858	0.5596	0.5959	0.6151	0.6338
	Hexp.	0.4590	0.4567	0.5009	0.5004	0.5103	0.4666	0.5060	0.5560	0.5762
		0.5489	0.5403	0.5403	0.5368	0.5700	0.5431	0.5604	0.6345	0.6420

Table 5 continued

S. no.	Population Locus	Beas 1	Satluj 2	Ganga 3	Yamuna 4	Kosi 5	Tons 6	Gerua 7	Brahmaputra 8	Mahanadi 9
	A_n	3.7500	4.2500	4.8750	4.7500	5.1250	4.1250	5.3750	4.5000	4.6250
		4.6250	5.1250	5.5000	5.3750	5.8750	4.7500	6.1250	5.3750	5.3750

Before (row above) and after (row below) correction for null alleles

Hobs. observed heterozygosity

Hexp. expected heterozygosity

Table 6 Inbreeding Coefficient (F_{is}) values for eight polymorphic microsatellite loci in *Labeo dero* from nine riverine sites

S. no.	Population Locus	Beas 1	Satluj 2	Ganga 3	Yamuna 4	Kosi 5	Tons 6	Gerua 7	Brahmaputra 8	Mahanadi 9
1.	*MFW-1**	+0.531	+0.413	+0.273	+0.458	+0.166	+0.309	+0.359	+0.365	+0.372
		+0.013	+0.014	+0.057	+0.040	+0.031	+0.087	+0.021	+0.090	+0.075
2.	*MFW-15**	+0.283	+0.451	+0.251	+0.324	+0.261	+0.520	+0.174	+0.248	+0.210
		−0.007	−0.022	+0.016	−0.025	+0.034	+0.003	−0.003	+0.038	+0.023
3.	*MFW-17**	+0.572	+0.248	+0.214	+0.169	+0.234	+0.655	+0.095	+0.193	+0.372
		+0.017	+0.017	+0.040	+0.037	+0.023	+0.018	+0.001	+0.060	+0.048
4.	*MFW-24**	+1	+1	+0.484	−0.006	+0.494	−0.067	+0.261	+0.702	+0.189
		+0.003	+0.011	+0.003	−0.006	+0.003	−0.067	+0.008	+0.036	+0.027
5.	*MFW-26**	+0.597	+0.372	+0.547	+0.045	+0.328	+0.474	+0.480	+0.510	+0.400
		−0.022	−0.011	−0.012	+0.038	+0.046	−0.008	−0.000	+0.001	+0.124
6.	*Ca-12**	−0.042	+0.203	+0.020	+0.176	−0.062	+0.153	−0.126	+0.075	+0.302
		−0.102	+0.038	+0.020	+0.014	−0.113	+0.038	−0.189	−0.021	−0.004
7.	*R-12**	+0.598	+0.554	+0.625	+0.576	+0.562	+1.0000	+0.447	+0.667	+0.627
		+0.006	−0.001	−0.024	−0.003	−0.020	−0.000	−0.009	+0.008	−0.019
8.	*R-3**	−0.098	+0.262	−0.020	+0.224	−0.147	−0.118	−0.211	+0.255	+0.067
		−0.098	+0.002	−0.020	+0.009	−0.147	−0.118	−0.211	+0.052	+0.028

Before (row above) and after (row below) correction for null alleles

reported for several vertebrates [36] and plants [37], populations of restricted distribution often display slightly lower heterozygosity estimates than for populations of species with more widespread distribution. Among European cyprinids, the common, wide spread and opportunistic roach *Rutilus rutilus* (L.) exhibited high degree of variability (He = 0.097–0.124) [38] in contrast to the endemic and rare *Leuciscus* species (He = 0.000–0.057) and *Chondrostoma* species (He = 0.022–0.070) [39–41].

Genetic variability estimates for *L. dero* based on eight polymorphic microsatellite loci (heterozygosity 0.54–0.63; alleles per locus 4.6–6.3) after corrections for the null alleles, were found to be close to the values reported for most of the freshwater fishes (heterozygosity, 0.54; alleles per locus 9.1; 10).

The determination of inbreeding coefficient (F_{is}) through partitioning of genetic variability as suggested by Wright [42] and Weir et al. [27] has been widely used to determine if the population has excess or deficit of heterozygotes. Three microsatellite loci (*MFW1**, *MFW15** and *R12**) deviated significantly from HWE (after the corrections for the null alleles) after the probability levels were adjusted for sequential Bonferroni adjustments. The similar significance levels were obtained when the loci were subjected to more powerful score tests. The locus *MFW1** had +ve F_{is} values and a test of heterozygote deficiency confirmed that those populations had a significant deficiency of heterozygotes, at the loci. The heterozygote deficiency could be due to several factors such as inbreeding, non-random mating, reduction in effective breeding populations and existence of the subpopulations or Wahlund effect [27]. However, these phenomena should affect the whole genome of the organism, and not one or two loci as found here. Therefore, the two most possible explanations are the presence of non-amplifying alleles and selection phenomenon. The presence of null alleles supported by lower +ve F_{is} values (after correction for null alleles) indicated that HW

Table 7 P_{HW} and P_{score} values for eight polymorphic microsatellite loci in *Labeo dero* from nine riverine sites

S. no.	Population Locus	Beas 1	Satluj 2	Ganga 3	Yamuna 4	Kosi 5	Tons 6	Gerua 7	Brahmaputra 8	Mahanadi 9
1.	*MFW-1**									
	P_{HW}	0.0000**	0.0000**	0.0004**	0.0000**	0.1746	0.0284*	0.0001**	0.0000**	0.0002**
		0.0022*	0.2732	0.2010	0.0002**	0.8612	0.4978	0.8669	0.0546	0.1396
	P_{score}	0.0000**	0.0000**	0.0039*	0.0000**	0.0269*	0.0641	0.0000**	0.0026*	0.0003**
		0.4966	0.6327	0.5838	0.4514	0.4276	0.4179	0.5955	0.2922	0.2039
2.	*MFW-15**									
	P_{HW}	0.0000**	0.0000**	0.0000**	0.0000**	0.0043*	0.0200*	0.0021*	0.0111*	0.0304*
		0.0423*	0.0231*	0.0892	0.0001**	0.05320	0.4845	0.1876	0.5926	0.3473
	P_{score}	0.0150*	0.0000**	0.0000**	0.0000**	0.0066*	0.0116*	0.0072*	0.0071*	0.0278*
		0.5498	0.2205	0.6253	0.5188	0.3736	0.6033	0.5166	0.4876	0.4372
3.	*MFW-17**									
	P_{HW}	0.0000**	0.0024*	0.0100*	0.0803	0.0332*	0.0000**	0.4348	0.0407*	0.0026*
		0.0091*	0.3811	0.5759	0.6757	0.8903	0.4304	0.8774	0.5030	0.3058
	P_{score}	0.0000**	0.0003**	0.0118*	0.0255*	0.0002**	0.0002**	0.0935	0.0791	0.0008**
		0.6629	0.5882	0.6168	0.3935	0.5132	0.3528	0.6424	0.4246	0.5186
4.	*MFW-24**									
	P_{HW}	0.0067*	0.0073*	0.0059*	1.0000	0.0156*	1.0000	0.1398	0.0001**	0.0781*
		0.1366	0.1406	0.2509	1.0000	0.1704	1.0000	0.4974	0.0429*	0.4235
	P_{score}	0.0067*	0.0073*	0.0030*	0.9944	0.0154*	0.8402	0.1013	0.0001**	0.1117
		0.4334	0.4069	0.5791	0.9944	0.4858	0.8402	0.4974	0.2864	0.4154
5.	*MFW-26**									
	P_{HW}	0.0000**	0.0027*	0.0001**	0.1222	0.0023*	0.1598	0.0098*	0.0001**	0.0064*
		0.0229*	0.1749	0.1102	0.1926	0.2749	0.6043	0.2910	0.3270	0.2971
	P_{score}	0.0000**	0.0068*	0.0000**	0.3692	0.0113*	0.0811*	0.0050*	0.0018*	0.0535
		0.1143	0.4621	0.1830	0.3767	0.3226	0.6759	0.4599	0.5005	0.3546
6.	*Ca-12**									
	P_{HW}	0.1323	0.0032*	0.2120	0.0631*	0.0233*	0.4392	0.0109*	0.1405	0.0132*
		0.2885	01257	0.2118	0.9585	0.0643	0.8381	0.0466	0.5961	0.7365
	P_{score}	0.9148	0.0168*	0.3957	0.0007**	0.9549	0.0719	0.9958	0.0062*	0.0035*
		0.1462	0.3994	0.3959	0.5669	0.0911	0.2208	0.0184*	0.3513	0.5239
7.	*R-12**									
	P_{HW}	0.0000**	0.0000**	0.0000**	0.0000**	0.0000**	0.1111	0.0000**	0.0000**	0.0008**
		0.0693	0.0088*	0.0003**	0.0010*	0.1366	0.6190	0.0686	0.0165*	0.2901
	P_{score}	0.0000**	0.0000**	0.0000**	0.0005**	0.0000**	0.1111	0.0000**	0.0000**	0.0009**
		0.3699	0.5855	0.5691	0.5138	0.4014	0.6667	0.5709	0.2510	0.3324
8.	*R-3**									
	P_{HW}	0.7114	0.0015*	0.6688	0.0004**	0.6620	0.3368	0.2785	0.0108*	0.5470
		0.7114	0.5880	0.6698	0.2137	0.6473	0.3460	0.2788	0.4885	0.6963
	P_{score}	0.1410	0.0001**	0.2406	0.0000**	0.0712	0.2885	0.0092*	0.0124*	0.1371
		0.1410	0.4729	0.2312	0.8439	0.0728	0.2838	0.0069*	0.5026	0.2992

Before (row above) and after (row below) correction for null alleles

P_{HW} Probability value of significant deviation from Hardy–Weinberg equilibrium, P_{score} probability value of significant heterozygosity deficiency

* Significant ($P < 0.05$)

** Significant after Bonferroni adjustmet

Table 8 Bottleneck Analysis for *Labeo dero* from nine different collection sites analysed with allozyme and microsatellite markers

Population	Allozyme	Microsatellite	
	IAM	TPM	SMM
Beas	0.81250	0.74219	0.54688
		0.32031	0.23047
Satluj	0.56250	0.03711*	0.01953*
		0.03711*	0.02734*
Ganga	0.46875	0.01953*	0.00977*
		0.12500	0.03711*
Yamuna	0.43750	0.12500	0.01953*
		0.23047	0.12500
Kosi	0.56250	0.00977*	0.00391*
		0.00977	0.00391*
Tons	0.04688*	0.02734*	0.01367*
		0.03711	0.02734*
Gerua	0.56250	0.00586*	0.00391*
		0.01953*	0.00586*
Brahmaputra	1.00000	1.00000	0.54688
		0.47266	0.27344
Mahanadi	0.56250	0.38281	0.31250
		0.32031	0.12500

The value given are probabilities through Wilcoxon test

*$P < 0.05$

deviation may be due to non-amplifying alleles. The occurrence of null alleles has been regularly recorded as one of the explanations for the observed deficit of heterozygotes. The non-amplifying allele(s) have been reported with microsatellite loci [43]. Allozyme analysis also revealed deviation from HWE owing to deficit of heterozygotes at *Est 1**, *Est 2** and *XDH** loci at many of the studied sampling sites. Loci *MFW15** and *R12** exhibited HW disequilibrium associated with marginal excess of heterozygotes (Fis = −0.025 and −0.003, respectively). This observed marginal excess of heterozygotes at these loci may not be of significant concern or could be more likely to be due to uninterpreted sampling error. The heterozygote excess in one locality reported in carp, *Cirrhinus mrigala* was attributed to the local mixing with the farm escapes [44]. This explanation may not be applicable here as aquaculture of *L. dero* is not yet present at any area.

The F_{st} indicated the proportion of genetic variation that could be attributed to genetic differentiation processes between the co-specifics from two localities [41]. In the present study theta [26] has been used to compute the partitioning of genetic variation. Rivers Beas and Satluj (both from Indus River System) exhibited weak to moderate differentiation with respect to other north side tributaries of Ganga, like Yamuna, Kosi, Gerua and Ganga main channel whereas moderate to high genetic divergence between the Mahanadi, Tons and Jiabharali populations and to the population in tributaries of middle Himalayan Indus system was evident from the partitioning data at both the markers systems. Overall F_{st} for all samples combined was found to be 0.059 (allozyme) and 0.019 (microsatellite) loci. Thus, approximately 2.0% (microsatellite) to 5% (allozyme) of genetic variation was found to be caused by genetic differentiation in *L. dero* population. Ward [33] reviewed 49 freshwater fish species and observed the F_{st} ranging from 0 to 74% with a mean of 22.2%. The F_{st} proportion over all the populations in the present studies indicated low to moderate level of genetic differentiation in *L. dero* populations. The results of AMOVA analysis also confirmed to low levels of differentiation as most of the genetic variation at both the markers (approx. 95%) was due to variability within populations from the same geographical locations, while only a small proportion could be attributed to the divergence between the populations. This pattern of variation corresponded to that obtained in another Indian major carp, *Labeo calbasu* (unpublished) using the same marker systems.

Several evolutionary forces which include random genetic drift, migration, mutation and their mutual interactions act on the wild populations and influence the pattern of genetic differentiation [20]. Random genetic drift tends to cause genetic differentiation, after subpopulations are fragmented and gene flow between them is either reduced or absent. In general, *L. dero* is primarily known to be a Gangetic carp, inhabiting parts of rivers in foothill regions that have substrata having boulders or rocks etc. It is highly unlikely that in the present geographical scenario, the *L. dero* in the drainages have the chance of intermixing, as the drainages Beas with Satluj and Yamuna, Kosi, Gerua with Ganges, combine downstream in plains, distant downstream the regions where the fish inhabits. Prehistoric descriptions clearly point out the possibility that the *L. dero* in different river basins covered in the study could have common ancestral gene pool. The pattern of genetic differentiation presents a contrasting scenario, weak to lack of genetic differentiation between the rivers of Indus and those in middle Himalayas. These populations exhibited moderate to high level of genetic differentiation with *L. dero* from Jiabharali river in north eastern Assam Himalayas, Mahanadi and Tons (a southern side tributary of Ganges). River Mahanadi and river Tons are non Himalayan Rivers that flow through the central plateau of India. The carps entered India during Eocene with migration of Indo-Malayan fishes through Indo-Brahma River, flowing westward from Assam in North-East to the present-day Arabian Sea [45]. The migration of fishes that initiated during Eocene (60 mya) continued till dismemberment of Indo-Brahma River and formation of Indus, Ganga and Brahmaputra river systems in late Pleistocene. The rivers

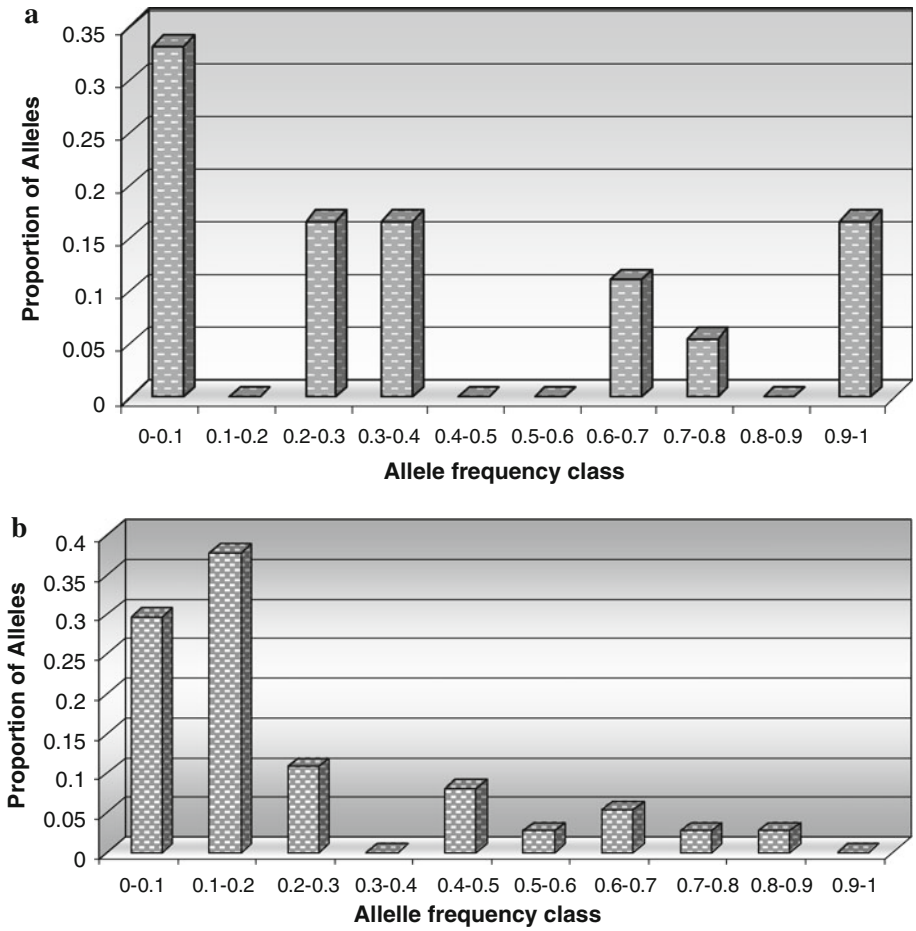

Fig. 2 Allele frequency distribution using microsatellite markers of *Labeo dero* (**a**) Mahanadi—before correction for null alleles, (**b**) Mahanadi—after correction for null alleles

Table 9 Genotypic homogeneity (*P*-value) at eight polymorphic allozyme and microsatellite loci in *Labeo dero*

Allozyme locus	*P*-value significance ($P < 0.005$)	Microsatellite locus	*P*-value significance ($P < 0.005$)
*EST-1**	<0.0001 S	*MFW-1**	0.0061 NS
*EST-2**	<0.0001 S	*MFW-15**	<0.0001 S
*EST-2**	<0.0001 S	*MFW-17**	<0.0001 S
*EST-2**	<0.0001 S	*MFW-24**	0.0001 S
*EST-2**	<0.0001 S	*MFW-26**	<0.0001 S
*GPDH**	<0.0001 S	Ca-*12**	<0.0001 S
*GPI**	0.3931 NS	*R-12**	0.0001 S
*XDH**	<0.0001 S	All	<0.0001 S
*PGDH**	<0.0001 S		
*PGM-2**	0.0088 S		
*MDH-2**	0.23 S		
All	<0.0001 S		

such as Satluj, Beas, Yamuna, Ghagara and other Himalayan rivers were formed as lateral rivers over course of time, due to faults occurring in Himalayan ranges. Dispersal of hill stream and torrent fishes was enabled by river capture, longitudinal river valleys and mountain faults. Such courses may change the course of streams without altering the torrential flow.

Notwithstanding fragmentation, the low genetic divergence could be due to possible gene flow between *L. dero* population in Indus and Ganges river systems through common flood plains and changes in the course of associated rivers. Remote sensing and archaeological evidence suggest that seasonal river basin Ghaggar located between Indus and Ganga basin is the remnant of the ancient mighty

Table 10 Pairwise comparison of F_{st} values (above diagonal) and probability values (below diagonal) in *Labeo dero* on the basis of allozyme analyses

FST	Beas	Satluj	Ganga	Yamuna	Kosi	Tons	Gerua	Jiabharali	Mahanadi
Beas	–	0.01496	0.02409	0.03991	0.01181	0.21235	0.0369	0.11755	0.11078
Satluj	0.034*	–	0.01965	0.04302	0.02793	0.20887	0.08899	0.15065	0.09959
Ganga	0.001**	0.003*	–	0.01463	0.00893	0.13413	0.06732	0.0868	0.04323
Yamuna	<0.0001**	<0.0001**	0.017*	–	0.00755	0.11341	0.04785	0.06051	0.02276
Kosi	0.057	0.006*	0.087	0.115	–	0.1268	0.01954	0.07834	0.04487
Tons	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**	–	0.21203	0.24492	0.13974
Gerua	0.001**	<0.0001**	<0.0001**	<0.0001**	0.015*	<0.0001**	–	0.06156	0.08501
Jiabharali	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**	<0.0001**	–	0.03218
Mahanadi	<0.0001**	<0.0001**	<0.0001**	0.04*	0.007*	<0.0001**	<0.0001**	0.009*	–

* Significant at $P < 0.05$

** Significant after sequential Bonferroni correction ($P < 0.002$)

Table 11 Pairwise comparison of F_{ST} values (above diagonal) and corrected F_{ST} values (below diagonal) in *Labeo dero* on the basis of microsatellite analysis

F_{ST}	Beas	Satluj	Ganga	Yamuna	Kosi	Tons	Gerua	Jiabharali	Mahanadi
Beas	–	0.00082	**0.01974**	**0.01676**	**0.00772**	**0.06292**	**0.00648**	**0.02699**	**0.09395**
Satluj	0.00507	–	**0.00576**	0.00529	0.00472	**0.06309**	0.00234	**0.02413**	**0.08104**
Ganga	**0.01819**	0.00737	–	0.00011	0.00446	**0.05365**	-0.00129	**0.00816**	**0.08144**
Yamuna	**0.02018**	0.00652	0.00557	–	0.00077	**0.06057**	0.0027	**0.01179**	**0.08022**
Kosi	0.01097	0.00697	0.00334	0.00716	–	**0.05758**	0.00158	**0.00766**	**0.07134**
Tons	**0.04609**	**0.05387**	**0.04196**	**0.05396**	**0.04937**	–	**0.04203**	0.02077	**0.14001**
Gerua	**0.00901**	0.00269	0.00107	0.00422	0.00292	**0.03874**	–	0.00521	**0.08579**
Jiabharali	**0.02262**	**0.02162**	0.00741	**0.02032**	0.00821	0.02342	0.00819	–	**0.07888**
Mahanadi	**0.06653**	**0.06103**	**0.06029**	**0.07342**	**0.05702**	**0.10048**	**0.06482**	**0.05210**	–

All significant ($P < 0.05$) values are represented in Bold

perennial river Saraswati with the present day Satluj River as its northwest tributary [46]. The changes occurred in the course of river Satluj to join Indus river system around 1900 BC [46], and Saraswati following the course of Yamuna in the Ganges. These changes can cause large scale mixing of putative subpopulations of aquatic organisms of different rivers and fragmentation of populations. Such gene flow could offset the divergence that random genetic drift might possibly cause [20]. The weak to lack of genetic differentiation in *L. dero* samples from Himalayan Rivers suggests that the *L. dero* in these rivers shared the genepool possibly at ancestral level. Moreover, it is possible that not much time may have lapsed after the fragmentation, for evolutionary forces to create detectable genetic differentiation. The fact that no private or locality specific allele is observed at any of the allozyme and microsatellite loci argues in favor of these reasons.

Lack of significant genetic divergence between *L. dero* of two Indus Rivers, Beas and Satluj was evident. The two rivers are not only in close vicinity (at certain places) but are linked through manmade channel connecting the two rivers in Himachal Pradesh. There is a possibility that gene flow could have been possible even in recent times. The observed moderate to high genetic divergence between the Mahanadi, Tons and Jiabharali populations and to the population in tributaries of middle Himalayan Indus system, clearly indicate the possibility of fragmentation of population which could have diverged over the course of time. Satpura hypothesis, proposed by Hora [47] explains the migration of hill stream and terrestrial fishes of Indo-Malayan affinity from Assam Himalayas to peninsular India, which occurred during Pleistocene. According to Satpura hypothesis, facilitation and interruption of migration of Indo-Malayan fishes to peninsular India was attributed to fluctuation in sea level during glacial (lowering of sea level) and interglacial (rise in sea level) period. The migration occurred through Garo Rajmahal gap, which became higher than sea level. Migrations were checked intermittently due to rise in the sea levels that isolated certain forms on hill tops. It is possible that species like *L. dero* radiated form Assam Himalayas during this migration to the rivers flowing from central plateau such as

Mahanadi, Tons etc. These rivers may be harboring the fragmented population which has undergone significant differentiation, evident from the allozyme and microsatellite data.

The study reveals the possibility of directional selection acting to increase differentiation in *L. dero*. Some studies have suggested that allozymes may be subjected to balancing selection, acting to decrease differentiation [48–50]. In contrast, Allendorf and Seeb [51] concluded that estimates of population structure produced by allozymes were generally comparable to those obtained with other markers, including microsatellites.

The general tendency for the lower genetic partitioning values using microsatellite rather than allozyme markers suggest the possibility that some factors may have acted to reduce apparent differentiation at the microsatellite loci. One possible explanation invoked previously for mammalian species is homoplasy generated by allele size constraints and high mutation rates at microsatellite loci [52]. Another possible explanation for the low microsatellite Fst might be the small sample sizes combined with large numbers of alleles also. But, this also seems untenable, because although this factor would certainly reduce statistical power, yet it should not reduce the estimate of the Fst parameter itself [53]. Moreover, when we treated the microsatellites as two-allele markers, combining all but the most common allele for each locus, to make their heterozygosities more comparable to the allozymes, the microsatellite Fst actually declined (recalculated Fst = 0.019). Finally, as noted above, selection is unlikely to have influenced the microsatellite results, particularly since it would have to be invoked for all of the microsatellite loci.

Data on allele frequency distribution based on Wilcoxon tests exhibited significant excess of heterozygotes in some rivers namely, Satluj, Kosi, Tons and Gerua. However, samples from river Mahanadi were found to possess genetic bottleneck using mode-shift criteria. Summarily, it is worth mentioning that the samples from these five rivers have undergone genetic bottleneck or reduction in their effective breeding population in recent generations.

The results provide evidence that *L. dero* in different rivers in India has distinct population substructure. In broad range, comparing across the riverine basins, the population of *L. dero* in Indus, Ganges, Brahmaputra and Mahanadi are genetically different. Within Ganges river system, *L. dero* in river Tons is genetically divergent from rest of the tributaries. There may be likelihood of presence of more than one genetic stock in Ganges, especially in associated rivers. Therefore, based on distribution of genetic differentiation detected by both markers, at least five different genetic stocks of *L. dero* across its natural distribution could be identified. In *L. dero*, rivers from of Indus river system i.e., Beas and Satluj form one genetic stock while in Ganges river system, samples of Ganga, Yamuna, Kosi and Gerua constitute another. Samples from rivers Tons and Jiabharali differentiated as separate stocks. *L. dero* samples of Mahanadi have exhibited adequate genetic divergence to be considered as a totally different genetic stock from samples other rivers studied. These rivers need adequate and conscious monitoring while rehabilitation programs are planned based on genetic stock structure.

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References

1. Chonder SL (1999) Biology of finfish and shellfish, 1st edn. SCSC Publishers India, Howrah, pp 166–169
2. Sehgal KL (1988) The ecology and fisheries of mountain streams of N.W. Himalayas. Thesis for the award of D.Sc. Degree, Meerut University
3. Sugunan VV (1997) Fisheries management of small water bodies in seven countries in Africa, Asia and Latin America. FAO fisheries circular no. 933. Rome, FAO, p 149
4. HPFD (2005) Himachal Pradesh Fisheries Department, Uttaranchal India. http://himachal.nic.in/fisheries/reservior.htm
5. Mahanta PC, Kapoor D, Dayal R, Ponniah AG (1994) Prioritization of the Indian fish species for conservation. In: Dehadrai PV, Das P, Verma SR (eds) Threatened fishes of India, Proceedings of the national seminar on endangered fishes of India, Nature Conservators, Muzaffarnagar, pp 377–386
6. Ferguson A, Taggart JB, Prodohl PA, McMeel O, Thompson C, Stone C, McGinnity P, Hynes RA (1995) The application of molecular markers to the study and conservation of fish populations with special reference to Salmo. J Fish Biol 47(1):103–126
7. Khuda-Bukhsh AR, Chanda T (1989) Somatic chromosomes of three species of hillstream fishes from Assam In: Das P, Jhingran AG (eds) Fish genetics in India, Proceedings of symposium on conservation and management of fish genetic resources of India, held on 11–13 April, 1986, pp 69–73
8. Nielsen EE, Hansen MM, Loeschcke V (1999) Genetic variation in time and space: microsatellite analysis of extinct and extant populations of Atlantic salmon. Evolution 53:261–268
9. Salini JP, Milton DA, Rahaman MJ, Hussein MG (2004) Allozyme and morphological variation throughout the geographic range of the tropical shad, hilsa *Tenualosa ilisha*. Fish Res 66: 53–69
10. DeWoody JA, Avise JC (2000) Microsatellite variation in marine, freshwater and anadromous fishes compared with other animals. J Fish Biol 56:461–473
11. Neff BD, Gross MR (2001) Microsatellite evolution in vertebrates: inference from AC dinucleotide repeats. Evolution 55(9): 1717–1733
12. Liu ZJ, Cordes JF (2004) DNA marker technologies and their applications in aquaculture genetics. Aquaculture 238:1–37
13. Mohindra V, Anshumala, Singh Rajeev K, Punia P, Kapoor D. Lal Kuldeep K (2005) Allozyme markers for population structure analysis in *Labeo dero* (Hamilton Buchanan, 1822). Asian Fish Sci 18(1–2):71–76
14. Mohindra V, Anshumala, Punia P, Narain L, Kapoor D, Lal KK (2005) Microsatellite loci to determine population structure of *Labeo dero* (cyprinidae). Aquat Living Resour 18:83–85

15. ECAFE (1966) A compendium of major international rivers in the ECAFE region. Water resources series, United Nation publication, no. 29
16. Whitmore DH (1990) Electrophoretic and isoelectric focusing techniques in fisheries management. CRC Press, Inc., Florida, p 350
17. Ruzzante DE, Taggart C, Cook D, Goddard S (1996) Genetic differentiation between inshore and offshore Atlantic cod (*Gadus morhua*) of Newfoundland: microsatellite DNA variation and antifreeze level. Can J Fish Aquat Sci 53:634–645
18. Wang D, Shi J, Carlson SR, Cregan PB, Ward RW, Diers BW (2003) A low-cast, high-throughput polyacrylamide gel electrophoresis system for genotyping with microsatellite DNA markers. Crop Sci 43:1828–1832
19. 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
20. Harlt DL, Clark AG (1997) Population substructure. In: Principles of population genetics, 3rd edn. Sinauer Associates, Inc., Sunderlands, Massachutetts, pp 111–159
21. Belkhir K, Borsa P, Goudet J, Chikhi L, Bonhomme F (1997) Genetics logicielsous windows pour Ia ge' ne' Tique des populations. http://www.Univ-montz.fr/(gentix/genetix/html), version 4.05.2
22. Chapuis MP, Estoup A (2006) FreeNA: microsatellite null alleles and estimation of population differentiation. Mol Biol Evol (submitted). chapuimp@ensam.inra.fr
23. Raymond M, Rouseet F (1995) An exact test for population differentiation. Evolution 49:1280–1283
24. Goudet J, Raymond M, de Meeus T, Rousset F (1996) Testing differentiation in diploid populations. Genetics 144:933–1940
25. Raymond M, Rousset F (1995) An exact test for population differentiation. Evolution 48:1280–1283
26. Wright S (1951) The genetical structure of populations. Ann Eugen 15:323–354
27. Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. Evolution 38:1358–1370
28. Excoffier LG, Laval G, Schneider S (2005) Arlequin ver. 3.0: an integrated software package for population genetics data analysis. Evol Bioinform Online 1:47–50
29. Lessios HA (1992) Testing electrophoretic data for agreement with Hardy–Weinberg expectations. Mar Biol 112:517–523
30. Cornuet JM, Luikart G, Piry S (1998) Bottleneck: description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. http://www.ensam.inra.fr/URLB
31. Spencer CC, Neigel EJ, Leberg LP (2000) Experimental evaluation of the usefulness of microsatellite DNA for detecting demographic bottlenecks. Mol Ecol 9:1517–1528
32. Sainudiin R, Durrett RT, Aquadro CF, Nielsen R (2004) Microsatellite mutation models: insight from a comparison of Humans and Chimpanzees. Genetics 168:383–395
33. 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
34. Ward RD, Woodwark M, Skinbinski DOF (1994) A comparison of genetic diversity levels in marine, freshwater and anadromous fishes. J Fish Biol 44:213–232
35. Nevo E (1978) Genetic variation in natural populations: patterns and theory. Theor Popul Biol 13:121–177
36. Nevo E, Beiles A, Ben-Shlomo R (1984) The evolutionary significance of genetic diversity: ecological, demographic and life history correlates. In: Mani GS (ed) Evolutionary dynamics of genetic diversity. Springer-Verlag, Berlin, Germany, pp 13–213
37. Frankham R (1996) Relationship of genetic variation to population size in wildlife. Conserv Biol 10:1500–1508
38. 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
39. Coelho MC (1992) Genetic differentiation of the Iberian cyprinids *Chondrostoma polylepis* steind, 1865 and Ch-willkommi steind, 1866. Arch Hydrobiol 125:487–498
40. Alves MJ, Coehlo MM (1994) Genetic variation and population subdivision of the endangered Iberian cyprinid *Chondrostoma lusitanicum*. J Fish Biol 44:627–637
41. Coelho MM, Brito RM, Oanbeco TR, Figueiredo D, Peris AM (1995) Genetic variation and divergence of Leuciscuspyrenaious and L. caroliterttii (Pisces, Cyprinidae). J Fish Biol 47(Suppl A):243–258
42. Wright S (1965) The interpretation of population structure by F-statistics with special regard to systems of mating. Evolution 19:395–420
43. Baranski M, Rourke M, Loughnan S, Austin C, Robinson N (2006) Isolation and characterization of 125 microsatellite DNA markers in the blacklip abalone, *Haliotis rubra*. Mol Ecol Notes 6:740–746
44. Chauhan T, Lal KK, Mohindra V, Singh RK, Punia P, Gopalakrishnan A, Sharma PC, Lakra WS (2007) Evaluating genetic differentiation in wild populations of the Indian major carp, *Cirrhinus mrigala* (Hamilton–Buchanan, 1882): evidence from allozyme and microsatellite markers. Aquaculture 269:135–149
45. Daniel RJR (2001) Endemic fishes of the Western Ghats and the Satpura hypothesis. Curr Sci 81:240–244
46. Lal BB (2002) The Saraswati flows on: the continuity of Indian culture. Aryan Books International, New Delhi, p 148
47. Hora SL (1949) Symposium on Satpura hypothesis of the distribution of Malayan fauna and flora to peninsular India. Proc Natl Inst Sci India 15:309–422
48. Karl SA, Avise JC (5053) Balancing selection at allozyme loci in oysters: implications from nuclear RFLPs. Science 256(5053): 100–102
49. Pogson GH, Mesa KA, Boutilier RG (1995) Genetic population structure and gene flow in the Atlantic cod, *Gadus morhua*: a comparison of allozyme and nuclear RFLP loci. Genetics 139: 375–385
50. Latta RG, Mitton JB (1997) A comparison of population differentiation across four classes of gene marker in limber pine (*Pinus flexilis* James). Genetics 146:1153–1163
51. Allendorf FW, Seeb LW (2000) Concordance of genetic divergence among Sockeye salmon populations at allozyme, nuclear DNA, and mitochondrial DNA markers. Evolution 54(2):640–651
52. Nauta MJ, Weissing FJ (1996) Constraints on allele size at microsatellite loci: implications for genetic differentiation. Genetics 143:1021–1032
53. Ellegren H (2000) Heterogeneous mutation processes in human microsatellite DNA sequences. Nat Genet 24:400–402