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NOTE

Microsatellite loci to determine population structure of *Labeo dero* (Cyprinidae)

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Abstract – Five polymorphic microsatellite DNA loci were identified in *Labeo dero* (Hamilton Buchanan), through cross-species amplification. Thirty-one primers developed for three cyprinids were tested in the study. The genetic variation detected at each polymorphic microsatellite locus was analysed. Significant genotype heterogeneity indicated that the samples are not drawn from same genepool. The identified microsatellite loci exhibited promise for use in fine scale population structure analysis of *L. dero*.

Key words: Microsatellite / Cross-priming / Genetic variation / Carp / *Labeo dero*

1 Introduction

Labeo dero (Cyprinidae) is naturally distributed all along foothill regions of the river systems originating from Himalayan range. It is important as a food fish and also has significance in the fishery of these regions. Categorized as a vulnerable fish (Mahanta et al. 1994), information on population structure of this species is essential to evolve conservation measures and management of fishery. Till now, there is no study to assess genetic variation in natural populations of *L. dero* and genetic markers have not yet been identified.

The microsatellites have been used to analyze genetic variation and population differentiation of variety of vertebrates including aquatic organism (Neff and Gross 2001). One of the characteristics of most of the microsatellite loci is that the sequences flanking microsatellites are conserved and there exists a potential to use these primers developed for one species to characterize loci in other related species (Zardoya et al. 1996). Many microsatellite primer sequences have been reported in Cyprinids (Zheng et al. 1995; Mohindra et al. 2001; Tong et al. 2002; Lal et al. 2004). The objective of the present study was to test, if primers developed for other cyprinids can provide amplification of microsatellite loci in *L. dero* with scorable pattern. The paper also evaluates the suitability of thus identified microsatellite loci in stock structure analysis of *L. dero*.

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2 Material and methods

2.1 Sampling sites and sample collection

The *L. dero* specimens were obtained through commercial catches, from three rivers, Satluj (Nangal, $n = 19$), Ganga ($n = 19$), Yamuna (Yamunanagar, $n = 19$). River Satluj is a part of Indus basin while rivers Yamuna and Ganga belong to Ganga basin (ECAFE 1966). Rivers Yamuna and Ganga do not join within the distribution range of *L. dero*. The blood samples collected through caudal puncture were fixed in 95% ethanol (1:5) and stored at 4 °C till use.

2.2 PCR amplification and electrophoresis

The total DNA was extracted following the procedure of Ruzzante et al. (1996). PCR amplification of each DNA sample was performed in a 25 μl reaction mixture, which contains 25−50 ng of DNA, 1X 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 0.5 pmoles 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. Amplified fragments were resolved on 10% non-denaturing PAGE with 1X TBE buffer for 5 h at a constant voltage of 150 V at 4−6 °C. The gels were silver stained (silver staining kit, Amersham Biosciences) and known DNA size marker (MspI cut PBR 322 DNA) was run in every gel. The size of the

Table 1. Characteristics of microsatellite loci amplified in *Labeo dero*.

S. No.	Locus in resource species	Primer sequence (5′ → 3′)	Repeat motif	Optimum Annealing temperature (°C)		Allele (*n*)	Quality of Product
				resource species	*L. dero*		
1	MFW1	GTCCAGACTGTCATCAGGAG GAGGTGTACACTGAGTCACGC	CA	55	57	4	S
2	MFW2	CACACCGGGCTACTGCAGAG GTGCAGTGCAGGCAGTTTGC	CA	55	59	1	F
3	MFW11	GCATTTGCCTTGATGGTTGTG TCGTCTGGTTTAGAGTGCTGC	CA	55	57	1	F
4	MFW15	CTCCTGTTTTGTTTTGTGAAA GTTCACAAGGTCATTTCCAGC	CA	55	51	4	S
5	MFW19	GAATCCTCCATCATGCAAAC CAAACTCCACATTGTGCC	CA	55	49	1	S
6	MFW17	CAACTACAGAAATTTCATC GAAATGGTACATGACCTCAAG	CA	55	51	4	S
7	MFW24	CTCCAGATTGCACATTATAG TACACACACGCAGAGCCTT	CA	55	51	2	S
8	MFW26	CCTGAGATAGAAACCACTG CACCATGCTTGGTGCAAAAG	CA	55	51	3	S

S = Good intensity bands, Scorable; F = Faint intensity bands.

amplified products was determined with ID Elite (Amersham Biosciences) software. The alleles were designated according to PCR product sizes and genotype of each individual was assigned manually.

2.3 Screening of primers and genetic diversity analysis

Microsatellite primers, twenty one from *Cyprinus carpio* (1, 2, 6, 7, 9, 11, 14, 15, 16, 17, 18, 19, 20, 23, 24, 26, 28, 29, 30, 31, 32) (Croojimans et al. 1997), five each from *Barbus barbus* (Barb37, 54, 59, 62, 79) (Chenuil et al. 1999) and *Catla catla* (*Ccat*G1, G2, A12, A7, C3) (Naish and Skinbiski 1998), were tested for amplification of homologous loci in *L. dero*. The three species are termed as resource species in the study. Four specimens (River-Yamuna collection) of *L. dero* were used in cross-species amplification experiment. The optimum annealing temperature was determined through experimental standardization for each primer pair. The primers yielding scorable amplified product, were again evaluated with larger sample size (57 individuals, from 3 rivers). Individual fish genotypes for each locus were determined. The allele frequencies, heterozygosity (observed and expected) values and mean no. of alleles per locus were calculated using software Genetix 4.02 (Belkhir et al. 1997). Tests for conformity to Hardy-Weinberg expectations (probability test) and genetic differentiation were done through Genepop 3.3d (Raymond and Rousset 1995).

3 Results and discussion

Out of 31 primer pairs tested for cross species amplification, successful amplification was achieved with eight primer pairs of *C. carpio* (Table 1). Primers from *B. barbus* and *C. catla* did not yield amplified product. The optimum annealing temperature, to get scorable band patterns, in *L. dero* differed from that reported for respective primer pair in resource species (Table 1).

The two loci (MFW2 and MFW11) yielded faint bands. The six scorable loci (MFW1, MFW15, MFW17, MFW19, MFW24 and MFW26) were assessed for detection of genetic variation using larger samples size of heterogeneous collections. Loci MFW1, MFW15, MFW17, MFW24 and MFW26 were polymorphic, while MFW19 was represented by only one allele (Table 1).

The number and size range of alleles at each locus together with heterozygosity values (observed $H_{obs.}$ and expected $H_{exp.}$) are given in Table 2. The data was analysed to assess allele frequencies conforming to that expected under Hardy-Weinberg equilibrium. No significant deviation was detected in the three sample sets at any of the locus ($p > 0.05$) after significant level adjusted for 10 simultaneous tests (Lessios 1992). The observed heterozygosity ranged from 0.265 (Satluj) to 0.402 (Ganga) and the mean number of alleles per locus varied between 2.6 (Satluj) to 3.2 (Ganga) (Table 2).

Test for genic differentiation over all samples was performed to test the hypothesis that samples have homogenous allele frequencies. Significant heterogeneity was observed ($p < 0.05$) at two loci (MFW1 and MFW15). Combined probability over all loci was 0.04 ($p < 0.05$), indicating that the three samples sets may not belong to single homogenous random mating population. G_{st} estimate for small sample size (Nei and Cheisure 1983) was 0.029, indicating that the 2.9% of the total observed variation is due to genetic differentiation. The various estimates provided strong evidence that the genetic variation detected at the identified microsatellite loci can be useful to depict population structure of *L. dero*. Nevertheless, larger sample size, including collections from other

Table 2. Parameters of genic difference for each population at each microsatellite locus in *Labeo dero*.

Locus	Riverine Site	Allele	Allele size range (bp)	H_0	H_e	Allelic Homogeneity (p)
MFW1	Satluj	3	169-181	0.352	0.610	0.029*
	Ganga	4	169-189	0.315	0.552	
	Yamuna	4	169-189	0.437	0.582	
MFW15	Satluj	3	150-160	0.277	0.331	0.037*
	Ganga	4	148-160	0.684	0.663	
	Yamuna	4	148-160	0.625	0.482	
MFW17	Satluj	4	198-214	0.375	0.326	0.170
	Ganga	4	198-214	0.722	0.521	
	Yamuna	3	204-214	0.615	0.544	
MFW24	Satluj	1	161	0.000	0.000	1.000
	Ganga	2	161-165	0.058	0.057	
	Yamuna	1	161	0.000	0.000	
MFW26	Satluj	2	114-110	0.556	0.054	0.496
	Ganga	3	114-106	0.117	0.212	
	Yamuna	2	114-110	0.066	0.064	
Mean over all loci	Satluj	2.60	-	0.212	0.265	0.043*
	Ganga	3.20	-	0.380	0.402	
	Yamuna	2.80	-	0.349	0.335	

geographic localities through the native range of the species, need to be analyzed to arrive at definite conclusion about the population structure of *L. dero*.

The present study successfully identifies five polymorphic microsatellite loci, that exhibit considerable promise as a means to discriminate stock structure in *L. dero*. This would be useful in the management of exploited stock as well as conservation of the species in rivers of fragile Himalayan ecosystem.

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References

Belkhir K., Borsa P., Goudet J., Chikhi L., Bonhomme F., 1997, GENETIX vers. 4.02, logiciel sous windows pour la génétique des populations, `http://www.univ-montp2.fr/~genetix/genetix.htm`.

Chenuil A., Galtier N., Berrebi P., 1999, A test of the hypothesis of an autopolyploid vs allopolyploid origin of a tetraploid lineage: applications to the genus *Barbus* (Cyprinidae). Heredity 82, 373-380.

Croojimans R.P.M.A., Bierbooms V.A.F., Komer J., van der Poel J.J., Gronen M.A.M., 1997, Microsatellite markers in common carp *(Cyprinus carpio*). Anim. Genet. 28, 129-134.

ECAFE, 1966, A compendium of major international rivers in the ECAFE region Water Resources Ser., United Nation, No. 29.

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.

Mahanta P.C., Kapoor D., Dayal R., Ponniah A.G., 1994, Prioritization of the Indian fish species for conservation. In: Dehadrai, P.V., Das, P., Verma, S.R (Eds.), Threatened Fishes of India; Proc. National Seminar on Endangered Fishes of India, Nature Conservators, pp. 377-386.

Mohindra V., Mishra A., Palanichamy M., Ponniah A.G., 2001, Cross-Species amplification of *Catla catla* microsatellite locus in *Labeo rohita*. Indian J. Fish. 48, 103-108.

Naish K.A., Skinbiski D.O.F., 1998, Tetranucleotide microsatellite loci for Indian major carps. J. Fish Biol. 53, 886-889.

Neff D.B., Gross M.R., 2001, Microsatellite evolution in vertebrates: inference from AC dinucleotide repeats. Evolution 9, 1717-1733.

Nei M., Chesser R.K., 1983, Estimation of fixation indices and gene diversities. Ann. Hum. Genet. 47, 253-259.

Raymond, M., Rousset, F., 1995, GENEPOP (vers. 1.2): A population genetics software for exact test and ecumenicism. J. Hered. 86, 248-249. vers. 3.3d available, hyperlink `http://www.cefe.cnrs-mop.fr/`

Ruzzante D.E., Taggart C.T., Cook D., 1996, Goddard S., Genetic differentiation between inshore and offshore Atlantic cod (*Gadus morhua*) of Newfoundland: microsatellite DNA variation and antifreeze level. Can. J. Fish. Aquat. Sci. 53, 634-635.

Tong-J., Wang-Z., Yu-X., Wu-Q., Chu-KH., 2002, Cross-species amplification in silver carp and bighead carp with microsatellite primers of common carp. Mol. Ecol. Notes 2, 245-247.

Zardoya R., Vollmer D.M., Craddock C., Streelman J.T., Karl S., Meyer A., 1996, Evolutionary conservation of microsatellite flanking regions and their use in resolving the phylogeny of cichlid fishes (Pisces: Perciformes). Proc. R. Soc. London B 263, 1589-1598.

Zheng W., Staeey N.E., Coffin J., Strobeck C., 1995, Isolation and characterization of microsatellite loci in the goldfish *Carassius auratus.* Mol. Ecol. 4, 791-792.