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Population structure analysis of *Labeo gonius* from three reservoirs of Uttarakhand using RAPD marker

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Population genetic structure of the fish, *Labeo gonius* from three reservoirs, i.e. Dhaura, Baigul and Nanak sagar, Uttarakhand, India was analysed by applying random amplified polymorphic DNA (RAPD) markers. RAPD–PCR products obtained using 15 decamer operon series (OPA, OPB, OPC and OPY) primers, were utilized for analysis of the population of *L. gonius* in each reservoir. Genetic observations based on percentage of polymorphic loci, the number of effective and observed alleles, Nei's genetic diversity and Shannon's information index indicated high level of genetic diversity in stocks of all three reservoirs of *L. gonius*, being more heterozygous in Nanak sagar followed by Baigul and Dhaura reservoirs. The percentage of genetic variation within (86.83) and among (13.17) all the stocks based on coefficient of gene differentiation (*Gst* = 0.131) indicated moderate genetic differentiation likely to be associated with small gene exchange responsible for weak sub-structuring of stocks in all three reservoirs.

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GENETIC variability within a stock/population of any fish species is valuable for its sustainable production. It is valuable for fitness of individuals in short-term perspective and in the long-term for survival of the population through adaptation to changing environmental conditions[1]. Poor fishery management, including excessive exploitation might result in depletion of the effective population leading to reduction of genetic diversity and ultimately the loss of gene pool[2,3]. Information on genetic structure of a population is necessary for identifying potential broodstocks, selective breeding programmes, stock enhancement for sustainable production and conservation of diversity. Random amplified polymorphic DNA (RAPD) analysis is based on polymerase chain reaction (PCR) amplification of discrete regions of the genome with short oligonucleotide primers of arbitrary sequences[4,5]. The method is simple, quick to perform and prior knowledge of the sequence of target DNA is not required[6]. It has been used extensively to detect genetic diversity for species and sub-species identification in guppy[7], tilapia[8], brown trout and Atlantic salmon[9], largemouth bass[10], ictalurid catfishes[11], seabass, seabream and mullet[12], analysis of population structure in black tiger shrimp[13] and marine algae[14], and analysis of genetic impact of environmental stressors[15]. It has also been successfully used for systematic and phylogenetic studies[16,17], population structure analysis, fishery management and conservation genetics of wild populations[18]. As intra-population genetic variation is essential for adaptation of species to environmental changes, the declining gene pool diversity from a population tends to lose genetic plasticity making it more susceptible to environmental changes and possibly leading to extinction[19]. The fish *L. gonius*, widely distributed in water bodies of North India, Asom and Odisha, and along the east coast up to the Krishna river in India, is a dominant species in different reservoirs of Uttarakhand. Three reservoirs, i.e. Dhaura (1200 ha), Baigul (2693 ha) and Nanak sagar (4262 ha) located in the tarai region of Uttarakhand, with different morpho-edaphic features, have self-recruiting populations of *L. gonius* as there is no stocking of this species from outside. It spawns during the southwest monsoon (July–August). These reservoirs are not connected with each other; hence the populations of *L. gonius* are distinctly isolated from each other for the last about 40 years. These reservoirs also have different patterns of sedimentation, water volume and abstraction, catchment area degradation, etc. Enhanced fishing activities during summer preceeding the breeding season associated with low water levels are likely to make effective population size of different fishes in these reservoirs highly vulnerable and more so in smaller ones. The present study was carried out to assess the genetic diversity in populations of *L. gonius* in the three above-mentioned reservoirs with the objective of devising appropriate management strategies for optimized sustainable production of this fish in these reservoirs.

Thirty-two specimens of *L. gonius* were collected from each of the reservoirs. Fin tissue samples were dissected out from caudal fin using sterilized scissors and forceps, preserved in ethanol and stored at –20°C before genomic DNA extraction.

Extraction of DNA from the fin tissue of each individual was performed by standard SDS-phenol/chloroform method[10]. The fin tissue samples were kept in 1 ml SET buffer (10 mM Tris-HCl, pH 8.0 containing 50 mm EDTA, 200 mm NaCl and 0.5% SDS) and digested with 0.5 µg/µl proteinase K at 55°C overnight. The resulting solution was centrifuged with phenol–chloroform–isoamyl alcohol in a refrigerated centrifuge (Sigma Centrifuges, USA). The supernatant was precipitated with ethanol and the precipitate was dissolved in 1× TE buffer. The quality and concentration of DNA were assessed by agarose gel electrophoresis.

Total 80 decamer primers, 20 from each series of OPA, OPB, OPC and OPY (Operon Technologies, Alameda, USA) were used. Thirty-five primers producing amplicons were selected for primary screening. On the basis of repeatability, sharpness and intensity of the bands, only 15 primers (see Table 1) were selected for further studies.

PCR was carried out following the standard RAPD protocol[20] with minor modifications. Genomic DNA was amplified in a reaction volume of 25 µl containing 10 mM Tris (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin, 5 pmol random primer, 1.25 mM each of dATP, dTTP, dGTP and dCTP, one unit *Taq* DNA polymerase and 25 ng template DNA. The reaction was performed in Gradient PCR mastercycler (Eppendorf, Germany) and programmed for 35 cycles of denaturation, annealing and extension. Template DNA was first

Table 1. Primers used in RAPD analysis of *Labeo gonius*

Primer code	Primer sequence	GC content (%)	Molecular weight (g/mol)
OPA-01	CAGGCCCTTC	70	2964
OPA-05	AGGGGTCTTG	60	3099
OPA-13	CAGCACCCAC	70	2942
OPA-14	TCTGTGCTGG	60	3050
OPA-15	TTCCGAACCC	60	2948
OPC-07	GTCCCGACGA	34	3013
OPC-08	TGGACCGGTG	34	3084
OPC-11	AAAGCTGCGG	32	3077
OPC-15	GACGGATCAG	32	3077
OPC-18	TGAGTGGGTG	32	3139
OPY-05	GGCTGCGACA	70	3053
OPY-13	GGGTCTCGGT	70	3075
OPY-14	GGTCGATCTG	60	3059
OPY-15	AGTCGCCCTT	60	2979
OPY-20	AGCCGTGGAA	60	3077

Table 2. Number and percentage of polymorphic loci (%*P*) in *L. gonius* stocks from all three reservoirs

Primer series	Population		
	Dhaura	Baigul	Nanak sagar
OPA	29 (70.73%)	36 (87.80%)	39 (95.12%)
OPC	27 (60.00%)	31 (73.81%)	41 (91.11%)
OPY	30 (66.67%)	39 (82.86%)	41 (97.62%)

denatured at 94°C for 4 min followed by 35 cycles comprising denaturation at 94°C, annealing at 36°C and extension at 72°C for 1 min each. After completing the cycles, the final extension step of 72°C was performed for 7 min. Approximately 10 µl of amplified products was separated by electrophoresis in 1.8% agarose gel at 4–5 v cm^{-1}, stained by ethidium bromide (10 mg/ml) and visualized under UV light in gel documentation unit (Alpha-Innotech).

Amplified fragments were scored as binary data, i.e. presence as 1 and absence as 0. Only data generated from reproducible bands were used for statistical analysis. The RAPD profile was computed using the software POPGENE version 1.31 and molecular weight of amplified bands was assessed by comparing with GeneRuler™ 1 kb DNA ladder (Fermentas Life Sciences). The DNA profiles generated for samples from all three stocks were compared and different parameters, i.e. percentage of polymorphic loci (%*P*), observed number of alleles (n_a), effective number of alleles (n_e), Nei's genetic diversity (H), Shannon information index (*I*), coefficient of genetic differentiation (*Gst*), gene flow (N_m), genetic distance and identity were computed to obtain intrapopulation and interpopulation genetic variation. Phylogenetic relationships among three populations of *L. gonius* were prepared following the unweighted pair group method (UPGMA) using arithmatic average method[21], implemented in PHYLIP[22] using POPGENE version 1.31 (ref. 23) based on available RAPD data. The binary data matrix was bootstrapped 1000 times (Winboot).

Totally 8344 consistently scorable polymorphic bands were generated with 15 RAPD primers in *L. gonius* from all three reservoirs. The wide size range of amplified fragments (450–3000 bp) was observed in all three stocks of *L. gonius*, which might be due to more priming site at the template DNA with the particular series of operon primers used. A similar range of fragment sizes has been observed by using RAPD markers in other teleosts belonging to the family Cichilidae, Mugilidae, Sparidae and Serranidae[12]. In the population genetic study employing RAPD technique, only 5 random primers produced 1344 amplicons in two different populations of cultured Korean catfish, *Silurus asotus*[24], while in another study on catfish 75 primers produced only 462 amplified fragments (200–1500 bp)[25]. This shows that the number and size of the fragments generated strictly depends upon the nucleotide sequence of the primer producing genome-specific fingerprints of random DNA fragments[26].

Percentage of polymorphic loci (Table 2), Shannon index, Nei's genetic diversity, number of observed and effective alleles (Table 3) indicate genetic diversity within the stocks of *L. gonius* in all three reservoirs.

Number and percentage of polymorphic loci with all 15 primers were observed to be highest in the stock of Nanak sagar followed by Baigul and Dhaura. The varied range of polymorphic loci (14.3–42.6%) in six *Labeo* species[27], and a very high polymorphic loci range (86.00–92.11%) were reported in five species of snappers[28] using RAPD markers. Preferential amplification of non-coding repetitive regions in the genome, eluding natural selection, is also reported to contribute higher percentage of polymorphism in several fishes[12,29,30].

Observed and effective number of alleles, Nei's genetic diversity and Shannon's information index indicated significant genetic variation within stocks of *L. gonius* from all three reservoirs. However, relatively lower genetic variability and a lower percentage of polymorphic loci in Dhaura stock followed by Baigul and Nanak sagar stocks might be correlated with the effective population size owing to fishing pattern in them. Maximum catch of *L. gonius*, indicating high fishing pressure on its population has been observed from Dhaura reservoir, which might be reducing its effective population size. Large areas of Dhaura get dried up in summer and fishes, including *L. gonius* are extensively exploited from deeper, isolated pockets holding them, thus adversely affecting their effective population size available for breeding in next season. Slightly lower Nei's genetic diversity values (0.2915 and 0.2167) in two populations of yellow grouper, *Epinephelus awoara* were correlated with smaller population size caused by overexploitation[31]. The problems of bottleneck drift and inbreeding, closely associated with small populations, have been correlated with effective population size (Ne) to population genetic structure of fishes[32]. Similar observations have also been made in two mahseers, i.e. *Tor khudree* and *Tor malabaricus*[33].

Observations related with genetic divergence in *L. gonius* stocks from all three reservoirs are presented in Table 4.

Small difference between values of population gene diversity (H_s) and total gene diversity (H_t) indicated moderate genetic differentiation among the stocks of *L. gonius*. Overall *Gst* value (0.1317) recorded for *L. gonius* suggested the possibility of less gene exchange among all the three stocks and indicated that 13.17% variation was attributable to interstock divergence, while 86.83% to individual differences within the stocks. Effective number of migrants per generation, an indicator of gene flow (N_m), at the observed level (1.344) is indicative of lower gene flow level compared to stocks with lower genetic differentiation. Similar level of genetic differentiation

Table 3. Genetic variability of *L. gonius* stocks in all three reservoirs (SD in parenthesis)

Population	Sample size	Observed number of alleles (n_a)	Effective number of alleles (n_e)	Nei's genetic diversity (H)	Shannon's information index (I)
Dhaura	32	1.7611 (0.3701)	1.4501 (0.3602)	0.2243 (0.1848)	0.3864 (0.2547)
Baigul	32	1.8610 (0.4176)	1.560 (0.3549)	0.2680 (0.1845)	0.4000 (0.2575)
Nanak sagar	32	1.9303 (0.2555)	1.7264 (0.2891)	0.3980 (0.1358)	0.5727 (0.1818)

Table 4. Nei's analysis of genetic diversity of *L. gonius* among all three stocks

Parameters	Value
Total genetic diversity in population (H_t)	0.3747 (0.0103)
Within-sample gene diversity (H_s)	0.3106 (0.0114)
Coefficient of genetic differentiation (*Gst*)	0.1317
Estimation of gene flow (N_m)	1.344

Table 5. Nei's unbiased measure of genetic identity (above diagonal) and genetic distance (below diagonal)

Population	Dhaura	Baigul	Nanak sagar
Dhaura	****	0.9508	0.7291
Baigul	0.1004	****	0.7542
Nanak sagar	0.1898	0.1345	****

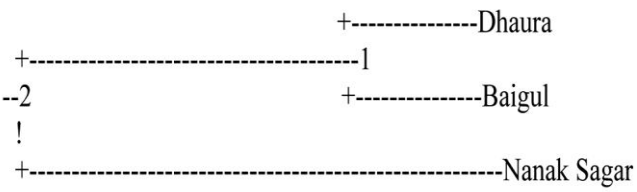

Figure 1. UPGMA dendrogram of *Labeo gonius* stocks from three reservoirs based on Nei's genetic distance.

was observed between two populations of *Eutropiichthys vacha* (*Gst* = 0.0958)[34] and *Brycon hilarii* (*Gst* = 0.108)[35] using RAPD markers. As RAPD bands arise from both coding and non-coding DNA regions, the regions of RAPD are supposed to be less responsive to selection and have higher tolerance to mutation making moderate to low differentiation rate common for it[5]. Geographical isolation, limited dispersal and phylopatric behaviour of populations are also responsible for promoting genetic differentiation, particularly in freshwater habitats[36].

Nei's[37] unbiased genetic identity and distance estimated between pairs of stocks of *L. gonius* for the three reservoirs (Table 5) reveal largest genetic distance between Dhaura and Nanak sagar stocks, and low genetic distance between Dhaura and Baigul stocks.

The UPGMA dendrogram (Figure 1) based on genetic distance measures also indicated clustering of Dhaura and Baigul stocks in one group, and Nanak sagar stock in separate group.

As genetic distance value increases with the increase in geographic distance, the observations pertaining to genetic distance in stocks of *L. gonius* seem to be correlated with the geographical distances among the stocks. The RAPD analysis of *Catla catla* from three rivers (Halda, Padma and Jamuna) and one hatchery population showed positive correlation between genetic and geographical distances[38]. Separate cluster for Nanak sagar indicated that stock of this reservoir is different from the other two stocks, which might be correlated with effective population size and geographical distance. As significant divergence in allele frequencies between populations has been reported to occur with up to 10 migrants per generation[39], the moderate level of genetic differentiation despite these reservoirs being well isolated from each other and fed by separate unconnected rivers indicated that the stocks of *L. gonius* in all three reservoirs are not strongly sub-structured.

The results of this study give a preliminary view of genetic variation both within and among the stocks of *L. gonius* from three reservoirs (Dhaura, Baigul and Nanak sagar) located in the tarai region of Uttarakhand. The available population genetic data indicate the presence of comparable genetic diversity in the stocks of *L. gonius* and moderate genetic differentiation among them because of little gene exchange. Maximum genetic variability observed in Nanak sagar stock followed by Baigul and Dhaura stocks might be correlated with the effective population size due to variable morpho-edaphic features, hydrologic regime and anthropogenic activities.

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