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Research Article

Low Heterozygosity and Gene Flow Obtained in Hatchery Raised Populations of Catla Catla (Ham, 1822) as Compared to Feral Population Identified Through DNA Fingerprinting

Garg RK*, Mishra V, Batav N and Singh RK
Centre of Excellence in Biotechnology, M.P. Council of Science and Technology, India

***Corresponding author:** RK Garg, Centre of Excellence in Biotechnology, Council of Science and Technology, Bhopal, Madyapradesh, India

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Abstract

The present investigation focused to revealed gene flow and genetic differentiation among riverine (Narmada river, n=04), reservoir (Tighra reservoir, n=04 and hatchery raised (Fish Federation Pond, n=07; Khatik Fish Farm, n=05) populations of *Catla Catla* (Hamilton, 1822) of Madhya Pradesh. Results clearly reflected as Riverine>Reservoir>Fish Federation Pond>Khatik Fish Farm gene flow and even all parameter analyzed for genetic divergence among the populations. Nei's gene diversity (h) was observed as 0.1382 in Fish Federation Pond, 0.1342 and 0.1739 in Khatik Fish Farm and Narmada River, 0.1490 populations reflecting much higher gene diversity in feral population. The Genetic Differentiation (GST) among the populations was found as GST=0.2380, estimates of gene flow between population (Nm=1.6010), intra-population heterozygosity as HS 0.2457 and total heterozygosity as HT=0.3225 clearly reflecting less genetic differentiation as overall when compared to other fish populations. Analyses genetic polymorphism (P) as 38.59% in hatchery raised population was obtained which as well much slighter as compared to wild populations since 60.30 in Narmada River and 51.63 in Tighra reservoir have been obtained. Overall research indicates that, as compared to wild stock, the genetic changes including reduced genetic diversity have taken place in hatched stocks. This baseline information on genetic variation would be useful for planning intended for effective strategies for conservation and remediation of *Catla Catla* freshwater fish species.

Keywords: Genetic divergence, Gene diversity (Hpop), Gene flow (Nm, GST), Genetic polymorphism (P), Genetic Differentiation (FST)

Introduction

Freshwater animals have been much greater losses than animals found in terrestrial ecosystems, and freshwater fishes are among the world's most endangered vertebrates [1]. Most of the fish used for human consumption is obtained through exploitation of wild populations. Allelic diversity (richness) is one of the most important and commonly used estimators of genetic diversity in populations. It strongly depends on the effective population size and past evolutionary history [2]. However, the number of observed alleles and their frequency distribution also depend on the sample size and the genetic marker system used. Thus, a practical method for reliable estimation of genetic diversity parameters in large populations is needed for population genetic studies and to develop scientifically sound strategies for genetic resource conservation. RAPD technique evaluates the genetic disparity within or between the taxa of concern by assessing the occurrence or lack of each product, which is directed by alteration in the DNA sequence at each locus [3].

Conservation biologists have to work in the face of continuously increasing anthropogenic pressures and inadequate resources for cataloguing biodiversity in the remaining near- pristine ecosystems. The decrease of biodiversity and limited resources for surveying it, have forced researchers to devise short- cuts for biodiversity surveys and conservation planning [4-6]. Small, geographically isolated populations are vulnerable to random demographic and environmental effects and typically have reduced levels of genetic diversity at the species [7-9]. Small populations are also more vulnerable to inbreeding depression and genetic drift, leading to reductions in genetic variability and decreased fitness. Furthermore, low levels of genetic variation in populations of rare species can potentially inhibit their ability to adapt to changing environmental conditions that can exacerbate risks of extinction [10-12].

The *Catla Catla* Fish is widely distributed in major rivers of India especially in gangetic river systems and their tributaries including major reservoirs [13,14]. It is good for food for economically weak communities due to low price in the market. It has enormous potential for high productivity and is known for its nutritive and therapeutic qualities. The present study endeavored to build upon previous studies on genetic structure and potential genetic effects of four stocking populations of *C. Catla* i.e., lotic habitat (Narmada river), lentic habitat (Tighra reservoir) and two man-made habitats (Fish Federation Pond and Khatik Fish Farm, Bhopal) for identification

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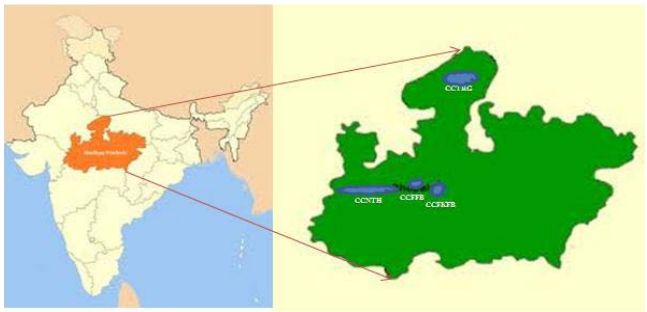

Figure 1: Map Samples collection sites i.e. Narmada River (CCNRH), Tighra Reservoir (CCTRG), Fish Federation Pond (CCFFB), Khatik Fish Farm (CCKFB).

Figure 2: Photographic representation of *Catla Catla* (Hamilton, 1822) collected from Narmada River, Tighra Reservoir Fish Federation Pond & Khatik Fish Farm.

of gene flow and hereditary traits depletion using nuclear DNA phenotypes variations. The *Catla* population is likely to be influenced by the degree of catchment connectivity, habitat obstruction and major geographic and ecological barriers. Therefore, once, we have the genetic structure and inferences regarding whether stocking genotypes has influenced the current genetic structure, then policy maker, ecologist may take necessary further action for conservation this population in their natural habitats.

Materials and Methods

Sample collection and genomic DNA extraction

Sampling sites were chosen to give a good representation of the distribution of this fish (Figure 1) in four different ecological niches i.e., riverine (Narmada river, n=04), reservoir (Tighra reservoir, n=04 and hatchery raised (Fish Federation Pond, n=07; Khatik Fish Farm, n=05) populations of *Catla Catla* of Madhya Pradesh (Figure 2). All Individuals were chosen at randomly and sampled directly from the water bodies using cast net and with the help of local fishermen (Table 1). The specimens were kept in the iceboxes and brought to the laboratory, muscle and liver tissues were obtained and finally stored at -80^{0}C. Total genomic DNA was isolated from these tissues using standard phenol-chloroform-isoamyl alcohol (25:24:1) method with some modifications and subsequently dissolved in 50 µl of E buffer [15].

Table 1: Details of the locations of *Catla Catla* genotypes sampled for DNA fingerprinting along with their coordinates.

Sample Code	Sampling Site	Geographical Coordinates	Samples Size (n)
CCNRH	Narmada River, Hoshangabad	22.75˚N 77.72˚	4
CCTRG	Tighra Reservoir, Gwalior	26˚13′17.11″N 78˚00′6.52″E	4
CCFFB	Fish Federation Pond, Bhopal	23.2084˚ N, 77.3790˚ E	7
CCKFB	Khatik Fish Farm, Bhopal	23.2437˚ N, 77.4731˚ E	5

PCR amplification and data collection

PCR amplification were carried out in 25 µl volumes, which contained 12.00 µl of Red Dye, 1.0 µl RAPD primer, 11.0 µl sterile water and 1.0 µl template DNA. Eight random primers RAn-01, RAn-02, RAn-03, RAn-04, RAn-06, RAn-07, RAn-09 and RAn-10 with accession numbers AM-765825, AM-773324, AM-765834, AM-750059, AM-765829, AM-773781, AM-750057 and AM-773782 respectively were used for final amplification of the DNA for fingerprinting. After preheating for 5 minutes at 94^{0}C, PCR was run for 45 cycles. It consisted of a 94^{0}C denaturation step (0.45 minutes), 37^{0}C annealing step (1 minute) and 72^{0}C elongation step (1.5 minutes) in a Thermal Cycler (Eppendorf, Germany). At the end of the run, a final extension period was appended at 72^{0}C for 7 minutes and then PCR products were stored at 4^{0}C until gel electrophoresis performed [15].

The amplified DNA fragments were separated on 1.2% agarose gel and stained with Ethidium Bromide. A low range DNA marker was run with each gel (100, 200, 300, 600, 1000, 1500, 2000, 2500 and 3000 bp, make Bangalore Genei, India). The amplified pattern was visualized on an UV trans-illuminator and photographed by a gel documentation system (Alpha-Innotech, USA) and after that scoring of the fingerprints and molecular weight was also performed by gel documentation system.

Bioinformatic Analyses for Population Genetic Estimation

The DNA fingerprints were scored as presence (1) or absence (0) of fragments on the gel photographs and RAPD fragments were compared among the *N. notopterus* populations. Observed Number of Alleles (na), effective number of alleles (ne), gene diversity (h), Shannon's Information Index (I), total number of loci and their percentage were estimated using a 'POPGENE' software ver 1.31 [16]. Gene flow (Nm), Intra-Population Heterozygosity (HS), Total Heterozygosity (HT), Relative Differentiation (GST), And Estimate Gene Flow (Nm) were also calculated to characterize the gene diversity and the distribution of the variation using 'POPGENE' program. Standard genetic distance, neighbour joining [17] tree was constructed using program 'MEGA' ver 5.0 [18] to determine the genetic relationship between hatchery raised and wild populations.

Results and Discussion

Genetic structure is mainly influenced by the combined effects of random genetic drift, restricted gene flow and differential selection pressure. The effects lead to low within and comparatively high among population genetic variation in species consisting of small and isolated populations [19]. Generally, when populations are close together, having possibility of enough gene exchange, there should be few differences in gene frequency, but if they are far apart, there

Table 2: Obtained quantity extracted DNA and further dilutions used for PCR amplification to obtained good and reproducible amplicons.

S. No.	Samples ID	Quality (260/280)	Concentration in ng/µl	Dilution Factor DNA:Water	Final volume used for PCR in ng/µl
A. Narmada River, Hoshangabad					
1	CCNRH-01	1.63	45.7	7:01	Final concentration of extraction genomic DNA was maintained as 40 ng/µl
2	CCNRH-02	2.04	166.8	1:03	
3	CCNRH-03	2.05	162.4	1:03	
4	CCNRH-04	1.61	250.3	1:05	
B. Tighra Reservoir, Gwalior					
1	CCTRG-01	2.04	277.6	1:06	Final concentration of extraction genomic DNA was maintained as 40 ng/µl
2	CCTRG-02	1.86	218.2	1:04	
3	CCTRG-03	1.17	54.2	3:01	
4	CCTRG-04	2.08	178.9	1:04	
C. Fish Federation Pond, Bhopal					
1	CCFFB-01	2.08	240.2	1:05	Final concentration of extraction genomic DNA was maintained as 40 ng/µl
2	CCFFB-02	1.99	548.3	1:12	
3	CCFFB-03	2.08	499.4	1:11	
4	CCFFB-04	2.09	223.3	01:05.5	
5	CCFFB-05	2.13	208.4	01:05.5	
6	CCFFB-06	2.06	2990	1:49	
7	CCFFB-07	1.99	2031.8	1:49	
D. Khatik Fish Farm, Bhopal					
1	CCKFB-01	2.08	123.41	1:02	Final concentration of extraction genomic DNA was maintained as 40 ng/µl
2	CCKFB-02	2.17	98.6	01:01.5	
3	CCKFB-03	2.19	81.7	1:01	
4	CCKFB-04	2.15	175.1	1:03	
5	CCKFB-05	1.99	348.9	1:07	

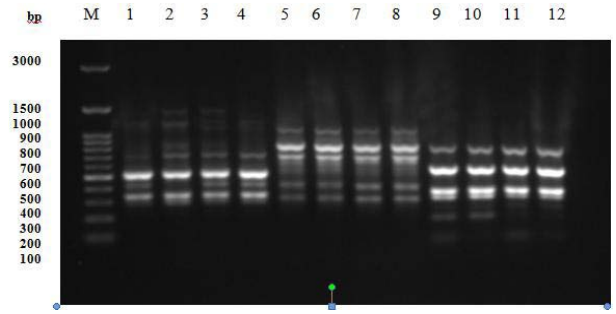

Figure 3: CCNRH and their DNA fingerprints obtained by RAn-03 (1-4), RAn-04 (5-8), RAn-06.

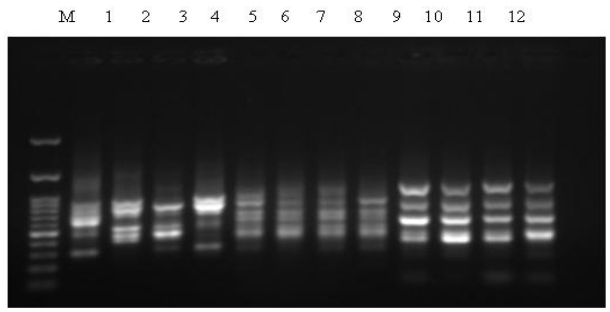

Figure 4: CCRG and their DNA fingerprints obtained by RAn-03 (1-4), RAn-04 (5-8), RAn-06.

should be strong differences [20].

The present study revealed moderate level of genetic variations between the 20 individuals collected from the four different ecological/habitat localities for 20 individuals. Hence, the genetic variability estimate might be due to restricted gene flow between each four populations except lotic ecological system. The amplification of DNA from *Catla Catla* genotypes using RAPD-PCR revealed a high number of bands and high polymorphism, both within and between four populations. Eight primers produced different classes of bands as high frequency polymorphic and polymorphic within only one or a few variant individuals. Some fragments, whilst polymorphic, were observed in the majority of samples.

The concentration of solution of nucleic acid can be determined by measuring the absorbance at 260 nm using a spectrophotometer. An A260 of 1.0 is equivalent to a concentration of 50 µg/µl for double standard DNA and 40 µg/µ1 for single standard DNA. If the A280 is also determined, the A¬260/A280 ratio indicates, if there are contaminants presents, such as residual phenol or protein. The A260/A280 ratio should be 1.8 for pure DNA and 2.0 for pure RNA preparations [21-23]. The quality and quantity of extracted genomic

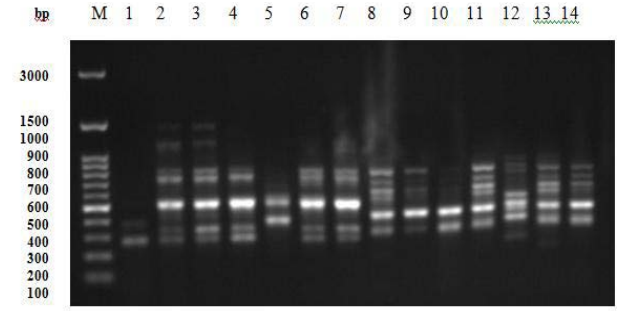

Figure 5: CCFB and their DNA fingerprints obtained by RAn-03 (1-7), RAn-04 (8-14).

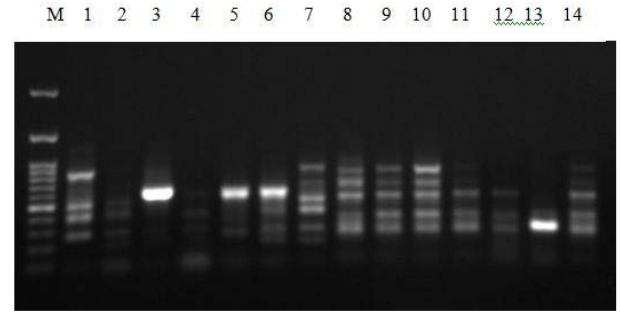

Figure 6: CCFB and their DNA fingerprints obtained by RAn-06 (1-7), RAn-07 (8-14).

DNA were checked by Nano drop UV-Vis Spectrophotometer (ND-1000, USA) and obtained 260/280 ratios range from 1.17 to 2.09 with concentration of ranges from 45.70 ng/µ1 to 2990.00 ng/µ1 (Table 2) as with similar with [24] were dilution as desired as 40.00 ng/µ1 for better PCR amplification.

The gene and genotypic frequencies data estimates that the supposition analyzed populations are in Hardy-Weinberg equilibrium and that the alleles from different loci do not co-migrate to a same gel position, where a visible fragment represents the dominant homozygous genotype and its absence represents the recessive homozygote [25-28]. Good random primer reproducibility were obtained the g-DNA of *Catla Catla* (Figure 3-6), DNA fingerprints analysis based random primers has been used to elucidate genetic diversity and polymorphism among and within four populations of Catla catla of Madhya Pradesh. Genetic Differentiation (GST) among the populations was found as 0.0454-0.85720±0.2380, estimates of gene flow between population as Nm= 0.0000-28.6205±1.6010, intra-population heterozygosity as HS= 0.0000-0.4002±0.12457 and total heterozygosity as HT= 0.0000-0.500±0.3225 clearly reflecting

Table 3: Nei's Original Measures of Genetic Identity and Genetic distance.

Population	Narmada River	Tighra Reservoir	Fish Federation Pond	Khatik Fish Farm
Narmada River	****	0.8433	0.8939	0.8842
Tighra Reservoir	0.1633	****	0.9107	0.8819
Fish Federation Pond	0.1122	0.0936	****	0.9204
Khatik Fish Farm	0.1231	0.1257	0.083	****

Note: Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

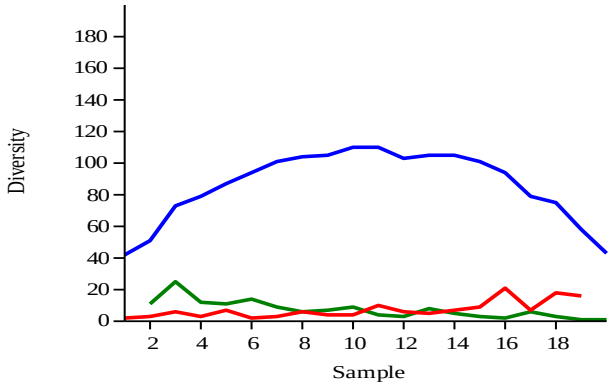

Figure 7: Diversity curve based on nuclear DNA phenotypes (■: Diversity, ■: Origination, ■: Extinction).

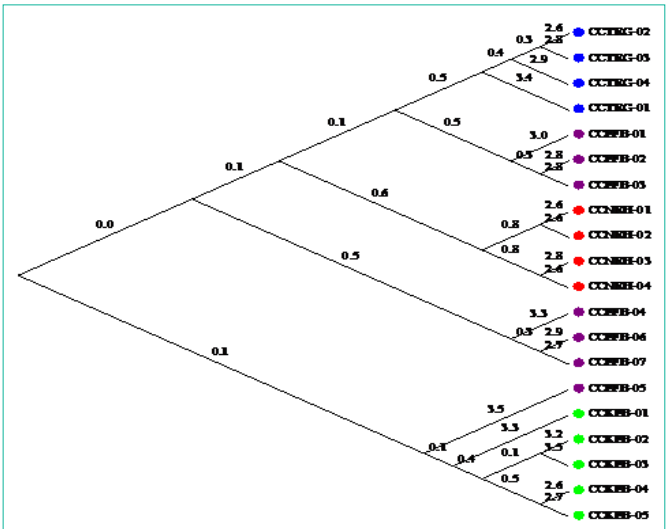

Figure 8: Jaccard's genetic distance based neighbor joining phylogenetic tree depicted for genetic relatedness among wild and cultured of Catla catla using RAPD-PCR DNA fingerprints.

Table 4: Genetic differentiation and gene flow among *Catla Catla* populations.

Parameters	Range of variations	Mean±SD
Intra-population (HS)	0.00-0.4002	0.2457±0.0069
Total Heterozygosity (HT)	0.00-0.500	0.3225±0.0133
Relative differentiation (GST)	0.0454-0.8572	0.238
Estimated gene flow (Nm)	0.00-28.6205	1.601

restricted genetic polymorphism among and between the populations (Table 3). High level of heterozygosity in detected in S. seenghala of Sutluj population (0.3935 as compared to Beas population (0.3556) may be the close proximity of the Sutlej collection site to the confluence where the two rivers meet which in turn leads to higher gene flow [29]. The expansion of the population niche and adaptations to new resources has theoretically been shown to be one of the causes of resource has theoretically been shown to ferent populations [30,31]. The Overall proportion of estimated gene flow (Nm) and Nei's gene diversity (Hpop) were 1.9369 and 0.266±0.147 obtained across the primers in the studied M. cavasius population [24]. Genetic variation in wild and hatchery raised population of *C. Catla* and he obtained

Table 5: Pattern of Gene diversity, allele information and genetic polymorphism in *Catla Catla* from 04 different ecological habitats.

Parameters	Fish Federation Pond (n=07)			Khatik Fish Farm (n=05)			Narmada River (n=04)			Tighra Reservoir (n=04)		
	Range of Variation	Mean	Standard Deviation	Range of Variation	Mean	Standard Deviation	Range of Variation	Mean	Standard Deviation	Range of Variation	Mean	Standard Deviation
Sample Size (n)	-	7	-	-	5	-	-	4	-	-	4	-
Observed number of alleles (na)	1.00-2.00	1.3859	0.4881	1.00-2.000	1.3859	0.4881	1.0-2.00	1.6033	0.4906	1.00-2.00	1.516	0.501
Effective number of alleles (ne)	1.00-2.00	1.2329	0.3318	1.00-2.000	1.2231	0.3286	1.00-1.990	1.2762	0.3172	1.00-1.978	1.235	0.303
Nei's (1973) gene diversity (h)	0.00-0.500	0.1382	0.1863	0.00-0.500	0.1342	0.1829	0.0-0.497	0.1739	0.1748	0.0-0.494	0.149	0.169
Shannon Information Index (I)	0.0-0.6931	0.2077	0.2727	0.0-0.6931	0.2029	0.268	0.0-0.690	0.2725	0.2538	0.0-0.687	0.235	0.25
Total number of loci	**184**			**184**			**184**			**184**		
Number of Polymorphic loci	**71**			**71**			**111**			**95**		
% of polymorphic loci (*P*)	**38.59%**			**38.59%**			**60.33%**			**51.63%**		

lowest percentage of Polymorphic loci (P) and gene diversity (Hpop) in hatchery raised population which may be to restricted gene flow due to cultured population (Table 5), whereas, wild stocks of *C. Catla* represent a diversified genetic resource and it maintains conserve and diverse gene pool [32]. The diversity curve based on nuclear DNA fingerprints was determined which showed rich diversity among the *Catla Catla* populations shown in (Figure 7).

Total numbers of multiple loci/DNA fingerprints were 184 in all four populations (Table 4) of which number of polymorphic loci were in 71 in Fish Federation and Khatik Fish Farm each, 111 in Narmada River, whereas, 95 loci were in Tighara Reservoir. However, population wise, the genetic analyses in Fish Federation and Khatik Fish Farm indicated lower genetic Polymorphism (P) as 38.59% and 38.59% as compared to rest of two populations i.e., in Narmada River 60.33% and Tighara Reservoir 51.63%. Nei's gene diversity (h) was observed as 0.00-0.50±0.1382 in Fish Federation, 0.00-0.50±0.1342 in Khatik Fish Farm, 0.00-0.4976±0.1739 in Narmada River and 0.00-0.4944±0.1498 in Tighara Reservoir. Similar observations on the other freshwater fishes were done by many scientists of India few of them are M. cavassius of two populations of which he obtained high polymorphism in Bansagar reservoir population has 98.86% [24] and 48.38% in Labeo rohita of Bangladesh [25].

Jaccard's and Euclidean dendrogram clearly distributed all the specimens in four clusters (Figure 3). The fishes of Narmada River are in one cluster, Tighra Reservoir in second cluster, Fish Federation Culture Pond in third cluster (CCFFB-01 to 03 only) and the fishes from Khatik Fish Farm in fourth cluster. Whereas, rest of 04 genotypes of Fish Federation genotypes made another cluster which not agreed with another 03 genotypes of nuclear DNA fingerprinting reproducibility's. Jaccard's and Euclidean dendrogram revealed that all 20 individuals of four locations are divided in clusters (Figure 8).

Genetic variability is an important characteristic population for the short term fitness of individuals as well as for long term survival of the population, permitting adaptation to the changing environmental conditions. The gene variation within population is extremely useful to gather the information on individual identity, breeding patterns, degree of relatedness and genetic variation among them is characterized for a population [26-35]. An overall research study indicates that, as compared to wild stock, the genetic changes including reduced genetic diversity have taken place in hatched stocks. This baseline information on genetic variation would be useful for planning intended for effective strategies for conservation and remediation of *C. Catla* species.

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