Genetic Analysis of Three River Populations of *Catla catla* (HAMILTON) Using Randomly Amplified Polymorphic DNA Markers

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ABSTRACT : The genetic variations in three major river populations viz. the Halda, the Jamuna and the Padma of the Indian major carp, *Catla catla* were analyzed by Random Amplified Polymorphic DNA (RAPD) markers. Four decamer primers were used for amplifying DNA of 10 individuals from each population. The proportion of polymorphic loci and the gene diversity estimates were 59.4 and 0.20 for the Halda, 37.5 and 0.14 for the Jamuna and 46.9 and 0.16 for the Padma populations respectively indicating the existence of a relatively high level of genetic variation in the Halda river population. The inter-population similarity indices, gene flow and genetic distance values indicated that the Jamuna-Padma population pair of catla was genetically closer than the Halda-Jamuna and the Halda-Padma population pairs in compliance with the geographical distances among them. The coefficient of gene differentiation (G_{ST}=0.13) reflects some degree of genetic differentiation among three populations of catla studied. The data suggest that the RAPD technique could be used to discriminate different river populations of catla. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 4 : 453-457)***

Key Words : RAPD, Polymorphic Loci, Genetic Variation, *Catla catla*

INTRODUCTION

The Indian major carp, catla (*Catla catla*, Hamilton 1822) is indigenous to the major river systems of Bangladesh, India and Pakistan. It is the fastest growing and second most commercially important freshwater fish in Bangladesh. Due to environmental modifications through siltation, dam construction and other anthropogenic activities, the opportunity of riverine fish to feed, navigate, and migrate and spawn has been constrained in the last two decades. Moreover, mercilessly catching the brood fish during the breeding time (April- May) by different fishing methods is claimed to contribute in the reduction of the carp hatchling production at the rate of 25-30 percent per year especially in the Halda river and as a consequence the supply of fry from natural sources is decreasing day by day (DoF, 2002). To overcome fish seed scarcity, a large number of hatcheries has been established which, at present, fulfill 99% of the total fish seed demand of the country (DoF, 2003). However, aquaculture practices and release of fry into natural water bodies also contribute to the species degradation, as inbreeding and hybridization are common practices in the hatcheries. In order to improve productivity of this fish, the hatchery stocks may need to be replaced partially or completely with better natural stocks. This is more important when the fish fry are produced to release in the open water bodies. It is however, essential to know the genetic structure of natural populations to be used as a founder or replacement stock so that any change in gene and genotype frequencies that may happen during the operation period can be assessed.

Genetic variation is useful for stock improvement breeding programs, management for suitable yield and conservation of diverse gene pool (Tassanakajon et al., 1997). Despite the commercial importance of the species, genetic data on catla stocks of Bangladesh are relatively scarce. Recently, allozyme electrophoresis on different stocks of Indian major carps including catla (Simonsen and co-workers, unpublished observation), RAPD analysis for four species of Indian major carp (Barman et al., 2003) and development of microsatellite loci for Indian major carp (Naish and Skibinski, 1998; McConnell et al., 2001) have been performed.

The RAPD technique is especially useful for revealing variation in species with low genetic variability when other techniques such as isozyme analyses and mitochondrial DNA control region sequencing cannot reveal differences among individuals (Welsh and McClelland, 1990; Williams et al., 1990; Bowditch et al., 1994). RAPD markers can be used for species and sex identification in animals (Appannavar et al., 2003; Huang et al., 2003). The present work deals with the genetic analysis of three river populations of catla to estimate genetic variation and relatedness among the populations using Random Amplified Polymorphic DNA (RAPD) analysis.

MATERIALS AND METHODS

Sample collection, DNA extraction, Primer selection, amplification and electrophoresis

Catla fry were collected from three major river systems viz. the Padma, the Jamuna and the Halda (Figure 1). Both the Padma and the Jamuna rivers flow from Himalayas and

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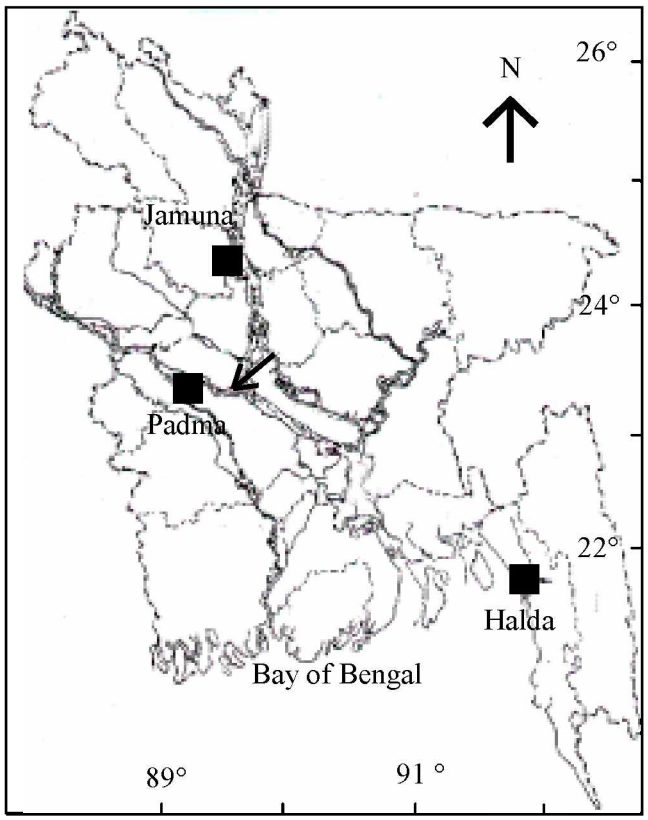

Figure 1. Map of Bangladesh showing sampling sites (■) of *C. catla*. The arrow indicates the joining point of the Jamuna and the Padma river.

pass a long distance (2,510 km and 2,900 km respectively) through India and Bangladesh and into the Bay of Bengal. In Bangladesh, both rivers join each other near the Goalanda upazilla of Rajbari district. On the other hand, Halda (approximately 35 km in length), an isolated freshwater tidal river originates in the hilly region of Chittagong district of Bangladesh and finally discharges into a relatively big river Karnaphuli. The fry collected from the rivers were reared in separate ponds at the field laboratory of Fisheries Faculty, Bangladesh Agricultural University, Mymensingh for 2 months. Finally, 10 fish from each river were sampled for RAPD analysis. Fin tissue was clipped from each fish and immediately placed in 95% ethanol.

The fin tissues were digested using Proteinase K, and genomic DNA was isolated using phenol: chloroform: isoamylalcohol extraction and ethanol precipitation following a standard protocol (Alam et al., 1996). In brief, approximately 30 mg fin tissue were taken and cut into small pieces. The tissue were then homogenized by using a micro homogenizer in extraction buffer (100 mM Tris.HCl, 10 mM EDTA, 250 mM NaCl and 1% SDS, pH=8). After homogenization 25 µl of 20 mg/ml Proteinase-K was added to the homogenate and incubated at 37°C overnight. The following morning the lysates were extracted once with equal volume of Phenol: Chloroform: Isoamyl Alcohol (25:24:1) and once with equal volume of Chloroform: Isoamyl Alcohol (24:1). DNA was precipitated using 0.6 volume of Isopropanol and then resuspended in TE buffer (10 mM Tris.HCl, 1 mM EDTA, pH=8). The concentrations of DNA samples were determined by a UV-spectrophotometer.

One kit (designated B), containing 20 decamer primers of random sequence, was obtained from Operon Technologies, Alameda, CA. Initially, the genomic DNA of one individual from each river sample was used as template for amplification of RAPD markers with each of the 20 decamer primers. Ten primers having high intensity of bands and no smearing were further used for amplification of DNA of two individuals from each population. A final subset of four primers (Table 1) exhibiting the highest quality banding patterns and sufficient variability for population analysis were taken for analysis of all the samples.

Experiments were run to optimize DNA, dNTPs and Taq DNA polymerase concentrations and to determine the optimum annealing temperature. Finally, PCR reactions were performed on each DNA sample in a 10 µl reaction mix containing 1 µl of 10×Taq polymerase buffer, 2 µl of 10 µM primer, 1 µl of 250 µM dNTPs, 1 unit of Taq DNA polymerase (GENEI, Bengalore, India) and 50 ng of genomic DNA and a suitable amount of sterile deionized water. DNA amplification was performed in an oil-free thermal cycler (Master Cycler Gradient, Eppendorf). The reaction mix was preheated at 94°C for 3 min followed by 40 cycles of 1 min denaturation at 94°C, 1 min annealing at 34°C and elongation or extension at 72°C for 2 min. After the last cycle, a final step of 7 min at 72°C was added to allow complete extension of all amplified fragments.

The amplified products were separated by electrophoresis on 1% agarose gel (Nacalai tesque, Inc. Kyoto, Japan) containing ethidium bromide in 1XTAE buffer at 120 V for 1½ h. Two molecular weight marker DNA, Lamda DNA-EcoT 14 I digest and 100 bp ladder were electrophoresed alongside the RAPD reactions. DNA

Table 1. Primers used to generate RAPD profiles from C. *catla* DNA

Primer	Sequence (5′-3′)	(G+C) %	Total scorable bands	Size range (bp)
OPB03	CATCCCCCTG	70	6	425-1,540
OPB08	GTCCACACGG	70	9	570-2,310
OPB09	TGGGGGACTC	70	6	460-2,630
OPB15	GGAGGGTGTT	60	11	475-1,790

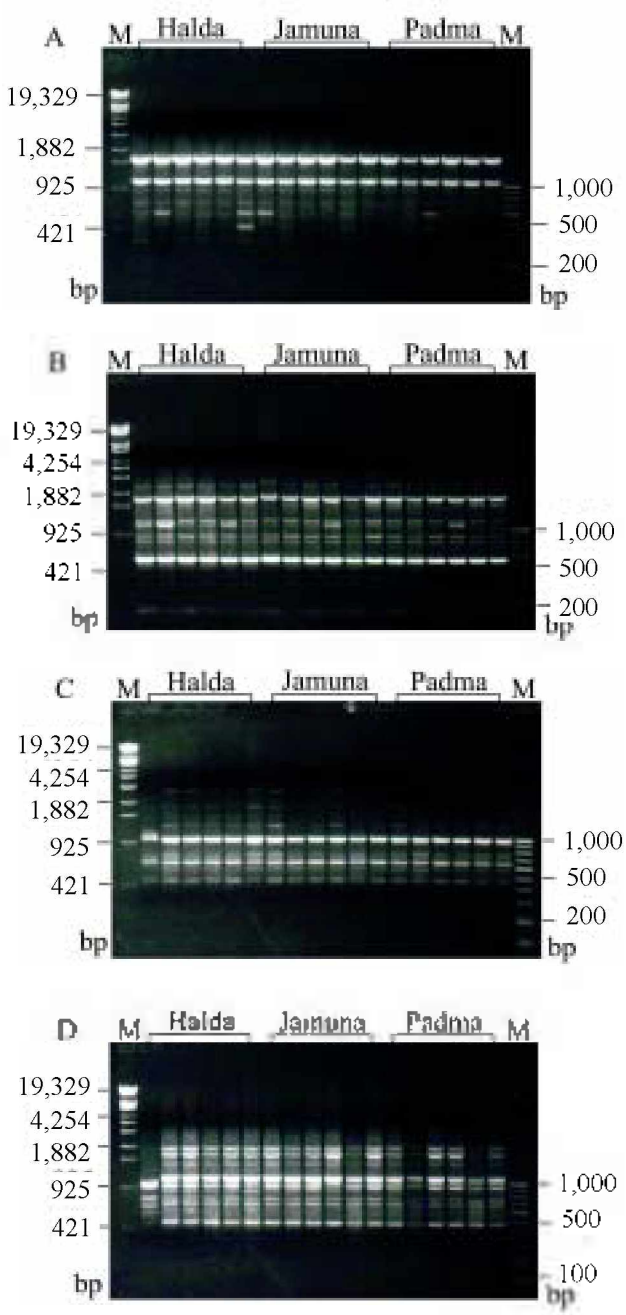

Figure 2. RAPD profiles of *C. catla* for the OPB-03 (A), OPB-08 (B), OPB-09 (C) and OPB-15 (D) primers of the Operon kit B. Ms are the molecular weight markers (Lamda DNA-EcoT14 I digest and 100 bp DNA ladder ladder).

bands were then observed on UV-transilluminator and photographed by a Gel Cam Polaroid camera.

RAPD data analysis

Fragments were scored as 1 if present and 0 if absent and the sizes of DNA fragments were measured by using the software. DNAfrag version 3.03 (Nash, 1991). The scores were then pooled for constructing a single data matrix. This was used for comparing the frequencies of all polymorphic RAPD markers and estimating Nei's (1973) gene diversity (h). gene flow (N_m). coefficient of gene differentiation (G_{ST}). genetic distance (D) and constricting a UPGMA (unweighted pair group method of arithmetic means) dendrogram among populations with 1000 simulated samples using POPGENE (version 1.31) (Yeh et al., 1999) computer program. Bandsharing based similarity indices between individuals within a population sample (S_i) and between population samples (S_{ij}) were calculated for all possible comparisons according to the method of Lynch (1991).

Table 2. Summary of band sharing based similarity indices within (S_i) (in parentheses), between (S_{ij}) (below diagonal) and gene flow (N_m value) (above diagonal) in the three studied populations of catla

	Halda	Jamuna	Padma
Halda	(87.7)	4.99	3.52
Jamuna	85.8	(88.2)	5.14
Padma	83.5	86.1	(87.7)

RESULTS

DNA profiles generated by RAPD primers

All the four primers produced different RAPD patterns. and the number of fragments amplified per primer varied. Among the primers OPB15 gave DNA profiles with more numerous bands than the other three primers (Table 1). The total number of fragments yielded from four primers were 32 of which 24 (75%) were polymorphic (the frequency of the most common allele is 0.95 or lesser) (Table 4). RAPD profiles obtained in this analysis are shown in Figure 2.

Intra- and inter-population polymorphisms

The values for intra-population similarity indices (S_i) were higher than the inter-population similarity indices (S_{ij}) (Table 2). The S_i values revealed no significant differences in diversity among the three populations of catla and the values were more or less similar (Table 2). The S_{ij} value between the Jamuna and the Padma population was the highest (86.1%) and the value between the Halda and the Padma was the lowest (83.5%) (Table 2).

The highest gene flow (N_m=5.14) was observed between the Padma and the Jamuna population whereas the lowest N_m (3.52) was found between the Halda and the Padma population (Table 2).

Frequencies of polymorphic RAPD markers are presented in Table 3. Two markers ($OPB15_{1,004}$ and $OPB15_{668}$) were found at a frequency of 0.051 in the Halda river population while absent from the other two rivers. Another marker ($OPB08_{1,354}$) was observed only in the Padma river sample at a frequency of 0.051. The proportion of polymorphic loci and Nei's (1973) gene diversity (h) values were found to be higher (59.4% and 0.20

Table 3. Frequencies of polymorphic RAPD markers in catla sampled from three river populations

RAPD markers	Halda	Jamuna	Padma
$OPB03_{920}$	0.684	0.368	0.051
$OPB03_{762}$	0.225	0.163	0.000
$OPB03_{653}$	0.106	0.225	0.106
$OPB03_{470}$	0.051	0.000	0.051
$OPB08_{2132}$	1.000	1.000	0.553
$OPB08_{1509}$	0.051	0.163	0.225
$OPB08_{1374}$	0.000	0.000	0.051
$OPB08_{1169}$	1.000	1.000	0.684
$OPB08_{1127}$	0.293	0.106	0.163
$OPB08_{870}$	1.000	0.684	1.000
$OPB09_{2590}$	0.684	0.452	0.368
$OPB09_{1291}$	0.553	0.452	0.368
$OPB09_{742}$	0.106	0.051	0.368
$OPB15_{1880}$	0.684	1.000	1.000
$OPB15_{1654}$	0.684	1.000	1.000
$OPB15_{1359}$	0.684	1.000	0.684
$OPB15_{1075}$	0.684	1.000	1.000
$OPB15_{1004}$	0.051	0.000	0.000
$OPB15_{938}$	0.684	1.000	1.000
$OPB15_{857}$	0.368	0.000	0.225
$OPB15_{766}$	1.000	0.684	0.684
$OPB15_{699}$	0.684	0.684	0.684
$OPB15_{668}$	0.051	0.000	0.000
$OPB15_{490}$	0.684	0.684	1.000

respectively) in the Halda population followed by those of the Padma population (46.9% and 0.16 respectively) whilst the values for the Jamuna river population were lower (37.5% and 0.14 respectively) (Table 4). The coefficient of gene differentiation among three different populations was found to be 0.13 (Table 4).

The values of pair-wise comparisons of Nei's (1978) unbiased genetic distance (D) between populations, computed from combined data for the four primers, ranged from 0.025 to 0.052. The Halda was the most genetically distinct population, which was segregated from the Padma population with the D value of 0.052 and from the Jamuna river population with the value of 0.029. The Padma and the Jamuna populations were separated from each other with the lowest genetic distance (D=0.025). Genetic differences between populations exhibited a consistent geographical pattern clearly illustrated in the UPGMA dendrogram based on Nei's genetic distance (Figure 3).

DISCUSSION

Report on genetic structure of different catla populations in Bangladesh is very scarce. It is also unknown how much the major river populations are genetically differentiated from each other. Recently, Simonsen and co-workers found nearly no intra- and inter-population variation in the three river and a hatchery population of catla by allozyme electrophoresis

Table 4. Estimates of genetic variations

Population	Number of polymorphic loci	Proportion of polymorphic loci (%)	Gene diversity (h)	Coefficient of gene differentiation (G_{ST})
Halda	19	59.4	0.20	
Jamuna	12	37.5	0.14	
Padma	15	46.9	0.16	
Combined population	24	75.0	0.19	0.13

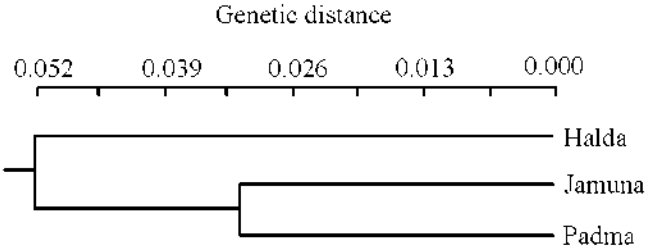

Figure 3. UPGMA dendrogram based on Nei's (1978) unbiased genetic distance, summarizing the data on differentiation between *C. catla* populations, according to RAPD analysis.

(unpublished observation). On the other hand, the RAPD method uncovered 24 polymorphic loci from four primers selected for population analysis. The result is consistent with the fact that the RAPD technique, being able to screen more easily a larger part of the nuclear genome than allozymes, may assess higher levels of genetic variation.

Analysis of the proportions of polymorphic loci, band-sharing based similarity indices for within-river samples and finally the gene diversity estimates indicate that a relatively high level of genetic variation exists in the Halda river population. The finding is consistent with the fact that the Halda river is a geographically isolated spawning ground of the major carps from where naturally spawned eggs of carps are collected. The geological structure of this freshwater tidal river makes the chances of mixing with other stocks impossible. The higher intra-river similarity and lower level of frequency of polymorphic loci and gene diversity estimates for the Jamuna or the Padma population could be an indication of comparatively closer relationship among individuals within each river system. In other words, both populations were more homogenous than that of the Halda river population. As expected, the band-sharing-based similarity indices are higher for within-river samples than for all between river sample comparisons. This implies that the catla population of one river is more homogenous than the combined group of the three populations. The higher between population similarities (S_{ij}=86.1%) and gene flow (N_m=5.14) between the Jamuna and the Padma populations over other two population pairs might be due to inter-connection between the two rivers (Figure 1). Moreover, both rivers are flood prone during rainy season and it is difficult to identify the demarcation line between

them, as they are over-flooded especially in the adjacent area of joining point of the two rivers. Therefore, there is a great possibility of mixing between the individuals of the two rivers. On the other hand, as the Halda river is located more distant from each of the other two rivers, the higher genetic differentiation between the Halda and the Jamuna or between the Halda and the Padma populations can be expected. Like the present study, Bielawski and Pumo (1997) discovered the divergence between the Hudson river and Roanoke river striped bass and concluded that the finding was not unexpected as these two river systems were geographically the most distant from each other. Nei's (1978) genetic distance was also used to evaluate the genetic variability and relatedness among catla populations. The results are consistent with the band-sharing based similarity indices. The greatest genetic distance exists between populations that are the most geographically distant. These results support the hypothesis that geographical distance is an important factor influencing the genetic relatedness of populations (Wright, 1943). The value of coefficient of gene differentiation (0.13) reflects some degree of genetic differentiation among three studied populations indicating the usefulness of RAPD markers in discriminating different populations of catla.

CONCLUSIONS

This study presents a first step towards the investigation of genetic variability among three different riverine stocks of catla by a DNA marker. The Halda stock is genetically more diversified than the Jamuna and the Padma population while the Jamuna and the Padma population are genetically more similar as they are geographically closer. Further studies involving large number of samples and primers need to be conducted to get more precise information about the genetic structure of the three riverine stocks of catla.

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