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Genetic differentiation of wild and hatchery populations of Indian major carp *Cirrhinus cirrhosus* in Bangladesh

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Abstract

Genetic differentiation of *Cirhinus cirrhosus*, three river (Halda, Jamuna and Padma) and six hatchery (Brahmaputra, Reliance, Kazi, Rupali, Bismillah and Pacific) populations covering a wide range of geographical distribution of this species were analyzed by allozyme electrophoresis. Four enzymes encoded by 7 loci were screened, where 3 loci were polymorphic (*Mdh-1*, Mdh-2*, Gpi-1**). The results showed that polymorphic loci per population (28.57%), mean proportion of heterozygous loci per individual (10.000), mean number of alleles per locus (1.286), and relative gene diversity (0.148) in river populations were higher than those for hatchery populations (23.81, 6.677, 1.238, 0.140, respectively). Also, the observed heterozygosity (*Ho*) and expected heterozygosity (*He*) in river populations (0.086 and 0.112, respectively) were higher than those of hatchery populations (0.046 and 0.106, respectively). The pairwise population differentiation (F_{ST}) values showed a lower level of genetic differentiation between hatchery and river population pairs. The unweighted pair-group method with arithmetic mean dendrogram of Nei's genetic distances showed relationship between genetic distance and geographic distance. Population were clustered into three groups: Halda, Padma and Jamuna river population in one group; Brahmaputra, Reliance and Kazi hatchery populations in second group and Rupali, Bismillah and Pacific hatchery population in third group. The present study revealed that there were obvious genetic variations among the natural and hatchery populations.

Key words

Allozyme electrophoresis, Allele frequency, Genetic diversity, Polymorphic loci

Introduction

Inbreeding along with crossbreeding, bottlenecks and negative selection is a common scenario in almost all hatcheries and hybrids are produced intentionally in the many carp hatcheries in Bangladesh (Simonsen *et al.*, 2005). Therefore, the genetic quality of hatchery originated seed has been questioned in aquaculture. Hatchery population, as a rule, should be replenished periodically by the riverine populations for genetic rejuvenation but if the riverine populations become genetically poor quality too, then there will be no place for betterment of the aquaculture stocks in future. Thus, there is an urgent need for knowing the genetic status of the riverine population as well as hatchery stocks of species cultured in Bangladesh.

Cirrhinus cirrhosus is one of the fast growing species among the principal Indian major carps employed in aquaculture in Bangladesh, India, Pakistan and Myanmar. The species are naturally available in Gangetic river system of India and Bangladesh (Talwar and Jhingran, 1991; Singh *et al.,* 2008). Because of its higher growth and good taste, mrigal is one of the most favorite species for pond culture in Bangladesh (Aung *et al.,* 2010; Ahsan *et al.,* 2013). It is stated that *C. cirrhosus* prone to loss of genetic diversity and variability in wild population because of escaping hatchery-reared fish in open waters (Aung *et al.,* 2010). However, till now natural fry have shown better growth

performance than hatchery produced fry (Islam and Alam, 2004; Nahar *et al.,* 2015, Siddik *et al.,* 2013).

Allozyme electrophoresis marker is used as an effective tool for fish population studies and fishery management (Aziz *et al.,* 2011; Islam and Hossain, 2012) and has been using to differentiate fish stocks that are genetically isolated to varying extents (Ayvazian *et al.,* 2004). In light of the above, the present study was carried out to assess genetic variation and relatedness in three river and six hatchery population of *C. cirrhosus* using allozyme markers.

Materials and Methods

Sample collection : The samples of *Cirrhinus cirrhosus* were collected from three main rivers (the Halda, the Jamuna and the Padma) and six hatcheries of three origins, (Brahmaputra and Reliance fish hatchery, Mymensingh; Rupali and Kazi fish hatchery, Jessore; Bismillah and Pacific fish hatchery, Comilla)(Fig. 1). Muscles were taken from each individual and stored at –18°C until electrophoretic analysis.

Allozyme electrophoresis : Horizontal starch gel electrophoresis and histochemical staining techniques were used to visualize different alleles according to Shaw and Prasad (1970). The enzymes including lactate dehydrogenase (LDH), malate dehydrogenase (MDH), phosphoglucomutase (PGM) and glucose phosphate isomerase (GPI) were analyzed for allozyme electrophoresis. Gel slices (1mm) were histochemically stained for different enzyme activities as outlined by Aebersold *et al.* (1987). Locus and allele designations were done following the standardized genetic nomenclature for protein coding loci (Shaklee *et al.,* 1990).

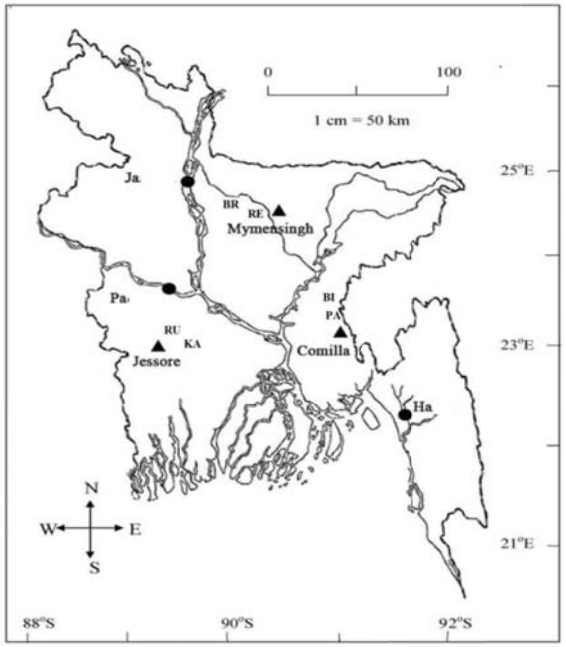

Fig. 1 : Map showing the collection sites of Mrigal *C. cirrhosus*. The populations are referred as Brahmaputra hatchery (BR), Reliance hatchery (RE), Rupali hatchery (RU), Kazi hatchery (KA), Bismillah hatchery (BI), Pacific hatchery (PA), Halda river (HR), Jamuna river (JA) and Padma river (PA)

Genetic data analysis : Allele frequencies were calculated simply by direct count of the proportion of different alleles. Distribution of observed genotypes were compared with the expected ones, calculated from Hardy-Weinberg (H-W) equilibrium using Chi-square (χ^2) test. Allele frequency and the analyses of Chi-square (χ^2) test were performed using Gene Alex (Peakall and Smouse, 2005) computer program (version 6.1). When the most common allele existed in frequency less than or equal to 0.95 at a given locus, the locus was regarded as a polymorphic. Mean proportions of polymorphic loci per population, mean number of alleles per loci and mean proportion of heterozygous loci per individuals, expected heterozygosity (*He*) and observed heterozygosity (*Ho*), Shannon's information index were analyzed with the help of POPGENE (Yeh *et al.,* 1999) computer package program (version 1.31). Genetic differentiation (F_{ST}) and gene flow (N_m) were measured with the help of Gene Alex (Peakall and Smouse, 2005) computer program (version 6.1). Based on Nei's genetic distance (D) (Nei, 1972), a dendrogram was constructed using unweighted pair group method of arithmetic average (UPGMA).

Results and Discussion

The electrophoretic patterns of *C. cirrhosus* indicated that enzymes were controlled by genes at seven presumptive loci. A maximum of two alleles (**a* and **b*) were found in *Mdh-1* Mdh-2** and *Gpi-1** loci with three genotypes (**aa*, **ab*, and **bb*). On an average 1.857 genotypes were produced by 1.429 alleles at seven loci (Table 1). Among them, only two loci (*Mdh-1** and *Gpi-1**) were found to be polymorphic in the Halda, Jamuna, Padma river and Brahmaputra hatchery population. Only *Gpi-1** locus were polymorphic in the Reliance and the Kazi hatchery. The

Table 1 : List of alleles and genotypes examined in *C. cirrhosus* populations

Locus	Alleles		Genotypes	
	No.	Type	No.	Type
Ldh-1*	1	*a,	1	*aa
Ldh-2*	1	*a,	1	*aa
Mdh-1*	2	*a,*b	3	*aa, *ab,* bb
Mdh-2*	2	*a, *b	3	*aa, *ab,*bb
Pgm*	1	*a	1	*aa
Gpi-1*	2	*a, *b	3	*aa, *ab,*bb
Gpi-2*	1	*a	1	*aa,
Average	**1.429**		**1.857**	

Rupali, Bismillah and Pacific hatchery population showed two polymorphic loci (*Mdh-2**and *Gpi-1**). The Jamuna river and the Brahmaputra hatchery population showed significant variation in allele frequency of *Mdh-1** locus. In *Mdh-2** locus allele frequency of the Bismillah, the Rupali and the Pacific hatchery populations showed significant variation. The Kazi hatchery and the Reliance hatchery populations showed significant variation in allele frequency of *Gpi-1** locus (Table 2).

Mean number of alleles per locus obtained in the present study was 1.286 and 1.238 in wild and hatchery populations, respectively, which was lower than that of 1.5 and 1.4 in hatchery and river populations, respectively in case of rohu, *Labeo rohita* (Alam and Khan, 2004). This may be due to small number of enzymes analyzed from relatively low sample size in the present study. For wild and hatchery populations of mrigal, the mean number of polymorphic loci per population was 28.57% and 23.81% (Table 3), which was lower than the reported average value (50%) based on allozyme studies in a hatchery population of *L. rohita* (Alam *et al.,* 2002) but higher than the value (27.3% and 23.5%) reported in natural and hatchery population of *L. rohita* (Khan and Alam, 2006). The polymorphic locus obtained from the present study was also higher than the value (24%) reported in natural population of *C. mrigala* through allozyme electrophoresis (Chauhan *et al.,* 2007). Suraiya *et al.* (2009) found that polymorphic locus in *Labeo bata* was 16.67% which was lower than that of the present study. Begum *et al.* (2013) recorded that the observed proportion of polymorphic loci in *L. gonius* ranged from 42.86% to 71.43% (average 60%). Tonny *et al.* (2012) studied genetic diversity between GIFT and GIFU using allozyme markers and estimated that polymorphic loci per population was 50%, which were considerably higher than the result obtained in the present experiment. Among the nine populations of mrigal, the mean percentage of polymorphic loci was higher in natural than in hatchery population due to natural population consists of diversified broodstock.

Mean number of heterozygous loci per individual in the present study was 10% and 6.777%, which was lower than the findings of Begum *et al.* (2013) reported mean number of heterozygous loci in *L. gonius* was 11.905%. The present study indicated that the value of heterozygous loci was lower than that reported by Alam *et al.* (2002). The average observed heterozygosity in wild and hatchery populations of *C. cirrhosus* was 0.086 and 0.046, respectively which is higher than the value (0.078) obtained by Simonsen *et al.* (2005) in case of mrigal populations of Halda river. The highest observed and expected heterozygosity (*Ho*= 0.071 and *He*= 0.061) was exhibited by the Jamuna river and Bismillah hatchery population which indicated that the gene pool might be maintained effectively.

Deviation from random mating within populations was measured by the inbreeding coefficient (F_{ST}). A negative value was found in the Rupali and the Pacific hatchery population indicated excess of homozygote. A positive value of inbreeding coefficient in three rivers and other four hatcheries indicated excess of heterozygote. Nei's (1972) analysis of gene diversity within population estimated genetic differentiation (F_{ST}) and gene

Table 2 : Allele frequency at seven presumptive loci of *C. cirrhosus* populations

Locus	Allele	Allele frequencies								
		Wild population			Hatchery population					
		1	2	3	4	5	6	7	8	9
*Ldh-1**	*a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*Ldh-2**	*a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*Mdh-1**	*a	0.825	0.875	0.850	0.825	1.000	1.000	1.000	1.000	1.000
	*b	0.175	0.125	0.150	0.175	-	-	-	-	-
P		0.456	0.101	0.003*	0.018*	-	-	-	-	-
λ^2		0.555	2.686	8.961	5.567	-	-	-	-	-
Fis		0.167	0.314	0.608	0.481	-	-	-	-	-
*Mdh-2**	*a	1.000	1.000	1.000	-	-		0.500	0.500	0.500
	*b				1.000	1.000	1.000	0.500	0.500	0.500
P								0.000*	0.000*	0.000*
λ^2								21.053	21.053	21.053
Fis								1.000	1.000	1.000
*Gpi-1**	*a	0.400	0.325	0.225	0.425	0.250	0.275	0.375	0.450	0.250
	*b	0.600	0.675	0.775	0.575	0.750	0.725	0.625	0.450	0.750
P		0.388	0.304	0.919	0.166	0.025*	0.000*	0.507	0.330	0.852
λ^2		0.743	1.056	0.010	1.922	5.032	16.677	0.440	0.948	0.035
Fis		0.134	0.254	0.004	0.284	0.467	0.875	-0.173	0.192	-0.067
*Gpi-2**	*a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
*Pgm**	*a	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

Table 3 : Genetic variabilities at seven presumptive loci of *C. cirrhosus* populations

	Population (wild ha^{-1})	Mean proportion of polymorphic loci per population (%)	Mean proportion of heterozygous loci per individual (%)	Mean no. of alleles per locus	Heterozygosity		Shannon's information index
					Ho	He	
Wild	1	28.57	10	1.286	0.093	0.113	0.162
	2	28.57	10	1.286	0.100	0.096	0.144
	3	28.57	10	1.286	0.064	0.128	0.137
	Mean	**28.57**	**10**	**1.286**	**0.086**	**0.112**	**0.148**
Hatchery	4	28.57	10	1.286	0.071	0.114	0.164
	5	14.29	5	1.143	0.029	0.055	0.080
	6	14.29	5	1.143	0.007	0.058	0.084
	7	28.57	10	1.286	0.057	0.135	0.186
	8	28.57	10	1.286	0.057	0.145	0.147
	9	28.57	10	1.286	0.057	0.128	0.179
	Mean	**23.81**	**6.677**	**1.238**	**0.046**	**0.106**	**0.140**

*P≤0.95, *Ho*, average heterozygosity observed; *He*, average heterozygosity expected.

flow (N_m) in nine population were 0.199 and 7.733, respectively. In pair-wise analysis, population differentiation (F_{ST}) value between Padma river and Reliance hatchery populations was highest (0.511) represents a high level of population differentiation. The overall F_{ST} value (0.199) of *C. cirrhosus* populations as obtained in the present study wa lower than the value (0.774) obtained for freshwater fishes such as loach (Khan and Arai, 2000) and Gobi (0.698) (Shimizu *et al.,* 2003). However, the present F_{ST} value indicated little genetic differentiation among the populations. The gene flow value (N_m) between the Padma and the Reliance hatchery populations was lowest (0.239) while that of the Halda and the Jamuna river populations was highest (43.885). Maximum genetic differentiation among different population pairs was observed between Padma river and Reliance hatchery population as hatchery was distantly located from the other and had no connection with each other.

Based on Nei's (1972) UPGMA dendrogram, nine populations of mrigal were divided into two major clusters by genetic distance of 0.179. Cluster-1 consisted of three populations and separated from each other by genetic distance of 0.999. Cluster-2 consisted of six populations and divided into two sub clusters and separated from each other by genetic distance of 0.0436. Subcluster-1 of cluster-2 consisted of Brahmaputra, Reliance and Kazi hatchery, and subcluster-2 was made by Rupali, Bismillah and Pacific hatchery populations and separated from each other by genetic distance of 0.0002. The observed genetic distance (*D*=0.179) among nine populations of *C. cirrhosus* in the present study was more or less similar to the findings of Pouyaud *et al.* (1998) who found average distances within the species pangasiid catfish (*D*=0.106) between population of Kalimantan and the population of Chao Phraya (*D*=0.145) between population of Teluk Kuantan in Sumatra and population of sole in Japan. The present *D* values were lower than the genus *Clarias* (*D*=0.366) reported by Na-Nakorn *et al.* (2002). The observed genetic distances (*D*= 0.014) reported by Alam, 2006 among the three populations of *P. pangasius* were lower than the present study. Based on UPGMA dendrogram, cluster between Padma and Jamuna river populations were formed due to connection of these river in Goalando. The Rupali and Bismillah hatchery formed same cluster might be due to same geographical region. Cluster between Reliance and Kazi hatchery populations were formed due to same allelic frequency. As genetic variation increased with increase in geographical distance, the observations pertaining to differentiation in stocks of *C. cirrhosus* seemed to be positively correlated with the geographical distances among the stocks.

Genetic variabilities obtained in the present study indicated that hatchery populations maintained lower genetic variation, which might have serious genetic impact on the natural stocks if these inferior carp fry were being stocked in open waterbodies.

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