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Comparison of Genetic Variability Between a Hatchery and a River Population of Rohu (*Labeo rohita*) by Allozyme Elctrophoresis

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Abstract: In order to compare the genetic variability of a hatchery and a wild population of rohu (*Labeo rohita*), four enzyme systems, PGM, GPI, MDH and LDH were studied using horizontal starch gel electrophoresis. The enzymes were found to be encoded by 6 gene loci: *Ldh-1*, Mdh-1*, Mdh-2*, Gpi-1*, Gpi-2** and *Pgm**. Within the six loci, *Gpi-2*, Mdh-1** and *Pgm** were found to be polymorphic. The mean number of alleles per locus and heterozygosity values (observed and expected) in wild population were 1.660 and 0.217 respectively which were higher than those observed in hatchery population (1.500 and 0.142 respectively). The relatively lower level of genetic variability of hatchery population could be attributed to a limited number of founders of the hatchery stock and inbreeding.

Key words: *Labeo rohita*, genetic variation, allozyme electrophoresis, wild population, hatchery population

Introduction

Rohu is the natural inhabitant of freshwater sections of the rivers of Bangladesh, India, Pakistan, Burma and the Terai region of Nepal (Jhingran and Pullin, 1985). Rohu has also been introduced in Sri Lanka. In Bangladesh, this species is mostly available in the Padma-Brahmaputra river system (i.e., Padma, Jamuna, Arial Khan, Kumar and Old Brahmaputra rivers) and in the Halda river system in Chittagong (Hussain and Mazid, 2001).

Fish hatcheries in Bangladesh play key roles in expanding aquaculture practices by providing fish seed for stocking. In year 2000, 187,343 kg fish spawn was produced in the hatcheries while only 2683 kg was collected from the natural sources, which testify the importance of hatchery stocks in aquaculture of this country. However, the performance of the hatchery-produced fry has been claimed to be declined significantly compared with the natural fry (Alam, M.S. and Shah, M.S., personal communication). The management of hatcheries in Bangladesh is mainly profit-driven. Little attention is paid to the quality of the fish seed produced in hatcheries. In order to keep the production cost at minimum, the hatchery operators usually maintain a less number of brood fish in their hatcheries and no genetic principle is followed in selecting and maintaining the brood fish. Therefore, it is not unlikely that the hatchery stocks have become inbred and the decline in general performance of the hatchery stocks, might be attributed to inbreeding depression, negative selection and unplanned hybridization.

Allozyme electrophoresis is one of the most powerful tools to study the genetic variability of a population. Variations in isozymes has been used for differentiating tetraploids from diploids of rohu, *L. rohita* (Sarangi and Mandal, 1996), for detecting hybrids between *L. calbasu* and *Cirrhinus mrigala* (Ahmed and Singh, 1985) and between *L. rohita* and *L. calbasu* (Krishnaja and Rege, 1977, 1979) and for investigating gene expression at various life stages (Basu *et al.*, 1992, Srivastava *et al.*, 1998). No studies have been undertaken to compare the genetic variability of a hatchery stock of rohu with that of a wild population in Bangladesh. In this study, the genetic variability of a hatchery stock of rohu at some allozyme loci has been compared with that of a wild population.

Materials and Methods

Fish specimen: Fry of rohu were collected from two different sources: (1) the Jamuna river near Bahadurabad Ghat in Jamalpur and (2) the Brahmaputra hatchery at Mymensingh. The fry were reared separately in the nursery ponds of the Field Lab. of the Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh for a period of three months. In order to carry out the electrophoretic study, 20 individuals (fingerlings) were taken randomly from each population. The muscle and liver samples were taken from each individual and stored at −18°C until electrophoretic analysis.

Allozyme electrophoresis: Horizontal electrophoresis was performed using 12% hydrolyzed potato starch in two buffer systems for four enzymes (Table 1) following the method of Aebersold *et al.* (1987). Allelic variants were designed to their relative electrophoretic mobility; the most common allele was given the number *a** and alternative alleles were labeled according to their rates of migration relative to that of the most common allele.

Genetic data analysis: The allele frequencies were calculated directly from observed genotypes. The mean proportion of heterozygous loci per individual, mean proportions of polymorphic loci per population and average number of alleles per locus were calculated so as to show the extent of genetic variability for each population following the method of Nei (1978).

Results

The electrophoretic patterns of liver and muscle samples showed that the enzymes were encoded by the genes at 6 presumptive loci (Table 2). In two of the 6 loci, the *Mdh-1** and *Gpi-2**, two genotypes were produced by two alleles, **a* and **b*. At locus, *Pgm** two to four genotypes were produced by two to three alleles, **a, *b* and **c*. At these six loci, 1.833 genotypes were produced by 1.660 alleles on the average (Table 2).

Table 1: Enzymes examined and tissues used for electrophoresis from *L. rohita*

Enzyme	Abbreviation	Enzyme pattern	E.C. number	Buffer system	Tissue
Glucose-6-Phosphate isomerase	GPI	Dimer	5.3.1.9	CA 6.0 / CA 7.0	Liver / Muscle
Lactate dehydrogenase	LDH	Tetramer	1.1.1.27	CA 6.0 / CA 7.0	Liver / Muscle
Malate dehydrogenase	MDH	Dimer	1.1.1.37	CA 6.0 / CA 7.0	Liver / Muscle
Phosphoglucomutase	PGM	Monomer	5.4.2.2	CA 6.0 / CA 7.0	Liver / Muscle

Table 2: Number and types of alleles and genotypes at 6 loci in two populations of *L. rohita*

	Alleles		Genotypes	
Locus	No.	Types	No.	Types
*Gpi-1**	1	**a*	1	**aa*
*Gpi-2**	2	**a and *b*	2	**aa, *ab*
*Ldh-1**	1	**a*	1	**aa*
*Mdh-1**	2	**a and *b*	2	**aa, *ab*
*Mdh-2**	1	**a*	1	**aa*
*Pgm**	3	*a**, *b** *and**c*	4	**aa, *bb, *bc, *ab*
Average	1.6600		1.8330	

Table 3: Allele frequencies at 3 loci obtained from liver and muscle of *L. rohita*

Locus	Allele	Hatchery population	Wild population
*Gpi-2**	**a*	0.950	0.900
	**b*	0.050	0.100
*Mdh-1**	**a*	0.925	0.800
	**b*	0.075	0.200
*Pgm**	**a*	0.500	0.425
	**b*	0.5000	0.525
	**c*	-	0.050

Table 4: Genetic variability at 6 loci in two populations of *L. rohita*

Population	Mean proportion of polymorphic loci per population (%)	Mean proportion of heterozygous loci per individual (%)	Mean number of alleles per Locus	Heterozygosity		
				Observed (H_o)	Expected (H_e)	H_o/H_e
Hatchery	50 %	15 %	1.500	0.142	0.125	1.129
Wild	50 %	15 %	1.660	0.217	0.178	1.212

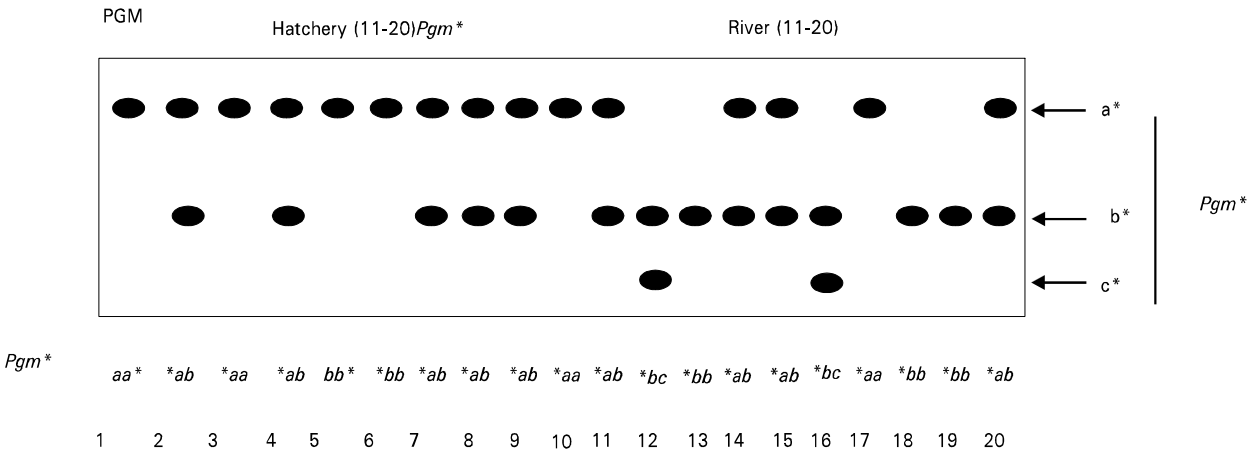

Fig. 1: Schematic representation of the electropherogram of phosphoglucomutase (PGM) showing the differences in genetic variability between the hatchery and wild population of *L. rohita*. Symbol *a**, *b** and *c** indicate three different alleles. The respective genotypes are shown below in each lane for a particular individual. The Arabic numbers below the panel indicate individual's identification number. Individuals 1-10 belong to the hatchery population and individuals 11-20 belong to the wild population. Note that individuals 1, 2, 4, 7, 8 and 9 from the hatchery population and 11, 12, 14, 15, 16 and 20 from the river population are heterozygous and a rare allele c* is seen in individuals 12 and 16 of river population.

The *Ldh-1**, *Gpi-1** and *Mdh-2** loci were found to be identically monomorphic and the *Pgm**, *Gpi-2** and *Mdh-1** loci were found to be polymorphic in both populations (Table 3). *Ldh-2** loci was not found in liver and muscle of either of the populations. A locus was considered as polymorphic if the frequency of the most common allele was equal to or less than 0.950. The extent of polymorphism varied among different loci. The wild population of rohu was found to be more polymorphic at locus *Pgm** compared with the hatchery population (Table 3).

The genetic variability of the wild population, as measured by the mean number of alleles per locus and heterozygosity values, was found to be higher than that of the hatchery population (Table 4). A rare allele *c** was observed in locus *Pgm** of the wild population which was absent in the hatchery population (Fig. 1).

Discussion

The present study was the first attempt to compare genetic variability in wild and hatchery populations of *L. rohita* in Bangladesh. The results presented here are based only on three polymorphic loci, which were confirmed by repeated allozyme electrophoresis and staining.

We screened five different enzyme systems in a wild and a hatchery population among which we could successfully interpret four enzymes namely GPI, LDH, MDH and PGM and found six different loci viz. *Ldh-1**, *Gpi-1**, *Gpi-2** *Mdh-1**, *Mdh-2** *and Pgm**. Unfortunately, we did not found *Ldh-2** locus may be due to small sample size.

In the hatchery population, frequencies of the most common allele *a** ranged from 0.500 (*Pgm**) to 0.950 (*Gpi-2**) while frequencies

of allele *b** ranged from 0.050 (*Gpi-2**) to 0.500(*Pgm**). These lower frequencies of allele *b** indicates that in hatchery population allele *b** is under threatened condition.

Presence of allele **c* with the frequency of 0.050 (*Pgm**) in the wild population is an important evidence of superiority of wild population compared with hatchery population in which allele **c* was completely absent (Fig. 1). Disappearance of allele **c* in hatchery population provides the evidence of the loss of genetic variability concomitant with the bottle neck effect.

The mean number of alleles per locus were 1.500 and 1.660 for the hatchery and the wild populations respectively. The value obtained from wild population of rohu in present study is lower than the value, (1.9) reported in natural population of common carp (Kohlmann and Petra, 1999). This may be due to a small number of enzymes analyzed from a relatively low sample size in this study.

Both in the river and hatchery populations, the number of polymorphic loci was 3, i.e. the mean proportion of polymorphic loci per population was 50% which is higher than the reported average polymorphic loci (12.5 to 25%) from 12 cultured stocks belonging to two races of common carp (Macaranas *et al.*, 1986), 40% polymorphic loci from Estonia common carp populations (Paaver and Gross, 1991). Kohlmann and Petra (1999) found 50% polymorphic loci in natural population and 25% in hatchery population of common carp.

However, compared with wild population slightly lower genetic variability was expected from hatchery population. In spite of our expectation, relatively higher value was obtained from hatchery population, which indicates several presumptions about the stock. Firstly, the population of this particular hatchery may consist of diversified parental stocks. It is possible as the hatchery operators, in case of shortage, buy brood fish of unknown origin from other farmers. Secondly, hybridization is a common phenomenon in the hatchery and Indian major carps can produce reciprocal fertile hybrids (Krishnaja and Rege, 1977 and 1979). However, as the hatchery operators do not maintain any record, they could not provide as with any information about the history of their hatchery stock.

In hatchery population, observed heterozygosity (H_o) and expected heterozygosity (H_e) are 0.142 and 0.125 respectively. However, in river population, the values are 0.217 and 0.178 respectively. All these values are somewhat higher than the cited values 0.176 in wild population and 0.089 in hatchery population of Atlantic Salmon (Crozier, 1997), average heterozygosity (H_o = 0.1100 ± 0.051; H_e = 0.1310 ± 0.0530) estimated in loach (Khan, 1998) and 0.000 to 0.154 ± 0.052 in Ciprinid genus *Notropis* (Avise, 1977). These higher values may be due to small sample size and limited number of enzymes analyzed the present study.

The present work gives preliminary information about the genetic structure of a wild and a hatchery population of rohu. It is expected that further genetic study will be conducted involving more samples and more enzymatic loci to compare the genetic status of the wild and hatchery populations of the important fish species.

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