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Allozyme variation in hatchery populations of silver and bighead carps in Bangladesh

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ABSTRACT

To elucidate allozyme variation, silver (*Hypophthalmichthys molitrix*) and bighead (*Aristichthys nobilis*) carps of three hatcheries were analyzed using seven enzymes and five of them showed clear banding pattern producing ten presumptive loci. Among the ten loci, *Mdh-1** in silver carp and *Mdh-2** in bighead carp populations were found to be polymorphic. The mean proportion of polymorphic loci per population (8.33±7.22%) and mean proportion of heterozygous loci per individual (2.22±1.92%) were higher in silver carp than in bighead carp populations (3.33±5.77% and 1.11±1.92%, respectively). UPGMA dendrogram showed that Maskanda and Parbatipur population made one cluster and separated from Brahmaputra in both silver and bighead carps by the *D* value of 0.016 and 0.05, respectively. These results indicate that the loss of genetic diversity in the existing stocks of silver and bighead carp populations might be due to use of small number of founder stock and it needs to be further restocked in Bangladesh from their original sources.

Introduction

Aquaculture in Bangladesh revolves around the cultivation of indigenous and exotic carps. The exotic carps, mostly Chinese carps have been introduced in Bangladesh since 1960. Among the Chinese carps, silver carp (*H. molitrix*) was introduced from Hong Kong in 1969 and China in 1994; and bighead carp (*A. nobilis*) from Nepal (1981) and China (1994) (Penman *et al.*, 2002 a). Domestication of these carps has been initiated with their commercial propagation in the late 1950s in China. The growth of progenies of wild silver and bighead carps has been detected 5-10% faster than those of hatchery stocks when the number of generations were produced using the same brood stocks through induced breeding in hatchery systems (Li *et al.*, 1987, 1990). Natural stocks of most of the Chinese carps grow faster in the Yangtze River (Li *et al.*, 1987). Both the carps are commonly used in polyculture especially with Indian major carps and Thai pangas (*Pangasius hypophthalmus*). Because of rapid growth and high market demand, it is accepted as an important contribution of aquaculture in Bangladesh. These species have also been introduced worldwide to improve water quality and to increase fish production, both in cultivatory facilities

and natural systems. At present about 890 hatcheries in Bangladesh produce fish seeds for the culture of freshwater species mainly carps including silver and bighead carps. The hatchery produced fish seeds might deteriorate the quality due to anthropological effects. After introduction of these two carps, hatchery owners produced the hybrid generation between silver and bighead carps, and hybrids often get mixed with pure stock because of frequent flooding in Bangladesh. The founder stocks may have been of poor or unknown quality which leads to poor performance through low genetic variation or a genetic makeup which is unrepresentative of the parent population (Penman *et al*., 2002 b). Also the scarcity of either mature silver or bighead carp males towards the end of the breeding season enforced the hatchery owners to produce hybrid progenies. If these F_1 hybrids are considered a parental stock for the next generation, there is a possibility of gene introgression between silver and bighead carps that might have negative consequences on the overall performance of these stocks. Similar genetic deterioration has been reported in the stocks of Indian major carps (IMCs) in Bangladesh (Khan, 2002). However, allozyme study was conducted to investigate the genetic variation of silver and bighead carps sampled from three hatcheries of Bangladesh.

Materials and methods

Fish specimens

Fry of silver and bighead carps were collected from three hatcheries of Bangladesh. The details about sampling are given in Table 1. The fry were reared in separate ponds to an average size of 70-80g so that muscle from individual fish could be taken for allozyme work. After rearing for around 3 months, the fish specimens were randomly caught and transported to the laboratory and stored at - 20°C for further use. Skeletal muscle was dissected and used for allozyme electrophoretic analysis without contamination.

Allozyme electrophoresis

The enzymes analyzed for enzyme pattern, E.C. number and abbreviation of enzymes used for horizontal starch-gel electrophoresis are shown in Table 2. Allozyme electrophoresis was conducted using muscle tissue with amine-citrate buffer (CA 6.1) system (Clayton and Tretiak, 1972). After electrophoresis, the gel slices (about 1 mm thickness) were histochemically stained for different enzymes as described by Shaw and Prasad (1970) with some modifications. Loci were numbered consecutively from the anodal to the cathodal side. Thus, the most anodal locus was designated as '1'. Gene nomenclature followed Shaklee *et al*. (1990). The electrophoretic bands corresponding to multiple alleles at each locus were alphabetically named as **a*, **b*, **c*, ———in the order of detection.

Genetic data analysis

Allele frequencies were calculated directly from observed genotypes. When the most common (major) allele existed in a frequency less than or equal to 0.95 at a given locus, the locus was regarded as polymorphic. The mean proportions of heterozygous loci per individual mean proportions of polymorphic loci per population and average number of alleles per population were calculated so as to show the extent of genetic variability for each population (Lewontin and Hubby, 1966). Expected and observed heterozygosity (*He* and *Ho*) were also calculated after Nei (1972) with the help of POPGENE (version 1.31) (Yeh *et al*., 1999) computer package program.

TABLE 1. *Silver* (H. molitrix) *and bighead carp* (A. nobilis) *populations used in the present study. Parenthesis shows the code name of each population.*

Population No.	Species	Sampling site	No.	Date of collection
1	Silver carp	Brahmaputra fish seed plant, Mymensingh [Brahmaputra silver carp]	30	June 8, 2003
2	”	Maskanda hatchery, Mymensingh [Maskanda silver carp]	”	February 18,2003
3	”	Parbatipur North West Fisheries Extension Project, Dinajpur [Parbatipur silver carp]	”	April 22, 2003
4	Bighead carp	Brahmaputra fish seed plant, Mymensingh [Brahmaputra bighead carp]	”	June 19,2003
5	”	Maskanda hatchery, Mymensingh [Maskanda bighead carp]	”	June 4, 2003
6	”	Parbatipur North West Fisheries Extension Project, Dinajpur [Parbatipur bighead carp]	”	April 22, 2003

TABLE 2. *Enzymes surveyed with resolution in muscle tissue of silver and bighead carps.*

Enzymes	Enzymes pattern	E.C. No.
Aspertate aminotransferase (AAT)	Dimer	2.6.6.1
Esterase (EST)	Monomer	3.1.1. -
Glycerol-3-phosphate dehydrogenase (G3PDH)	Dimer	1.1.1.8
Glucose-6-phosphate isomerase (GPI)	Dimer	5.3.1.9
Lactate dehydrogenase (LDH)	Tetramer	1.1.1.27
Malate dehydrogenase (MDH)	Dimer	1.1.1.37
Phosphoglucomutase (PGM)	Monomer	5.4.2.2

The preceeding analysis of allozyme data was performed using POPGENE, (version 1.32) (Yeh *et al.*, 1999) and G-Stat, (version 3.1) (Siegismund, 1995) and TREEVIEW (Roderick, 2000) package computer program. Based on the *D*-values, dendrogram was made by the unweighted pair group method using arithmetic average (UPGMA) method (Nei, 1978).

Results and discussion

Allozyme variation and genotypes

The enzymes were controlled by the genes at ten presumptive loci (*Est-1**, *Gpi-1**, *Gpi-2**, *Ldh-1**, *Ldh-2**, *Mdh-1**, *Mdh-2**, *Mdh-3**, *Mdh-4** and *Pgm**) in silver and bighead carp populations (Table 3). The *Mdh-1** locus in silver carps of Maskanda and Parbatipur populations were heterozygous (**a* and **b*) whereas Brahmaputra population was homozygous (**b*=1.00). The electrophoretic pattern of *Mdh-3** and *Mdh-4** loci were completely absent in the silver carp populations. The *Mdh-1** in bighead

TABLE 3. *Allele frequency at ten presumptive loci in silver and bighead carp populations.*

Locus	Allele	Allele frequency					
		Silver carp			Bighead carp		
		Brahma putra	Mask anda	Parba tipur	Brahm aputra	Mask anda	Parba tipur
*Est-1**	**a*	1.00	1.00	1.00	1.00	1.00	1.00
*Gpi-1**	**a*	1.00	1.00	1.00	1.00	1.00	1.00
*Gpi-2**	**a*	1.00	1.00	1.00	1.00	1.00	1.00
*Ldh-1**	**a*	1.00	1.00	1.00	1.00	1.00	1.00
*Ldh-2**	**a*	1.00	1.00	1.00	1.00	1.00	1.00
*Mdh-1**	**a*	-	0.40	0.50	1.00	1.00	1.00
	**b*	1.00	0.60	0.50	-	-	-
*Mdh-2**	**a*	1.00	1.00	1.00	0.28	1.00	1.00
	**b*	-	-	-	0.72	-	-
*Mdh-3**	**a*	-	-	-	1.00	1.00	1.00
*Mdh-4**	**a*	-	-	-	1.00	1.00	1.00
*Pgm**	**a*	1.00	1.00	1.00	1.00	1.00	1.00

carp populations presented no variation (**a*=1.00) and *Mdh-2** showed heterozygosity (**a* and **b*) in Brahmaputra population only (Table 3 and Fig. 1). The invariant allelic results from Brahmaputra in silver carp, and Maskanda and Parbatipur in bighead carp populations might be due to the use of small number of broods as founder stocks.

Genetic variability

The mean proportion of polymorphic loci per population of silver carp and bighead carp were respectively 8.33±7.22% and 3.33±5.77% (Table 4). This value is much lower than the reported range of of 11.8-23.5% on an average for silver carp and 29-40% for bighead carp based on allozyme studies in three Chinese river populations (Li *et al.*, 1990). Thus, the genetic variation decreases in the stocks of Bangladesh due to long chain breeding using small number of effective breeding broods and long time domestication of same stock. This conclusion is also suggested by the mean proportion of heterozygous loci 2.22±1.92% for silver carp and 1.11±1.92% for bighead carp population. The range of average heterozygosity in Chinese river sources of silver and bighead carp were respectively 4.84-5.11% and 10.42-11.33% (Li *et al.*, 1990). The polymorphism of the bighead hatchery population in the US was 4% (Brummett *et al.*, 1988).

The average number of alleles per locus was 1.08±0.07 in silver carp and in bighead carp was 1.03±0.06. (Table 4). The average observed heterozygosity (*Ho*) was 0.06±0.06 and 0.02±0.09 in silver and bighead carp, respectively. The average expected heterozygosity (*He*) was 0.04±0.04 and 0.01±0.02 in silver and bighead carp, respectively (Table 4). The heterozygosity ratio (*Ho/He*) was 1.98 in Parbatipur silver carp and 1.22 in Brahmaputra bighead carp indicated that the high values of heterozygosity in comparison to other populations might be due to inbreeding effect within a smaller base population.

Genetic differentiation

The genetic distance (*D*) varied from 0.00 to 0.17 among six populations of

TABLE 4. *Genetic variabilities at ten loci of silver and bighead carps.*

Population	Mean proportion of polymorphic loci* per population (%)	Mean proportion of heterozygous loci per individual (%)	Mean no. of alleles per locus	Heterozygosity			
				Ho	*He*	*Ho/He*	1-*Ho/He*
Brahmaputra silver carp	0.00	0.00	1.00	0.00	0.00	0.00	0.00
Maskanda silver carp	12.5	3.33	1.13	0.06	0.06	0.95	0.05
Parbatipur silver carp	12.5	3.33	1.13	0.13	0.06	1.98	-0.98
Mean±SD	8.33±7.22	2.22±1.92	1.08±0.07	0.06±0.06	0.04±0.04	0.98±0.99	0.35±0.55
Brahmaputra bighead carp	10.0	3.33	1.10	0.05	0.041	1.22	-0.219
Maskanda bighead carp	0.00	0.00	1.00	0.00	0.00	0.00	0.00
Parbatipur bighead carp	0.00	0.00	1.00	0.00	0.00	0.00	0.00
Mean±SD	3.33±5.77	1.11±1.92	1.03±0.06	0.02±0.09	0.01±0.02	0.41±0.70	0.07±0.13

TABLE 5. *Estimated genetic distances among six hatchery populations of silver and bighead carps based on ten loci. The genetic distances, D=0.00 showed between Parbatipur and Maskanda bighead carp whereas same populations of silver carp showed D=0.0006.*

Population No.	Population	1	2	3	4	5	6
1.	Brahmaputra silver carp						
2.	Maskanda silver carp	0.0160					
3.	Parbatipur silver carp	0.0254	0.0006				
4.	Brahmaputra bighead carp	0.1674	0.0955	0.0830			
5.	Maskanda bighead carp	0.1054	0.0371	0.0254	0.0535		

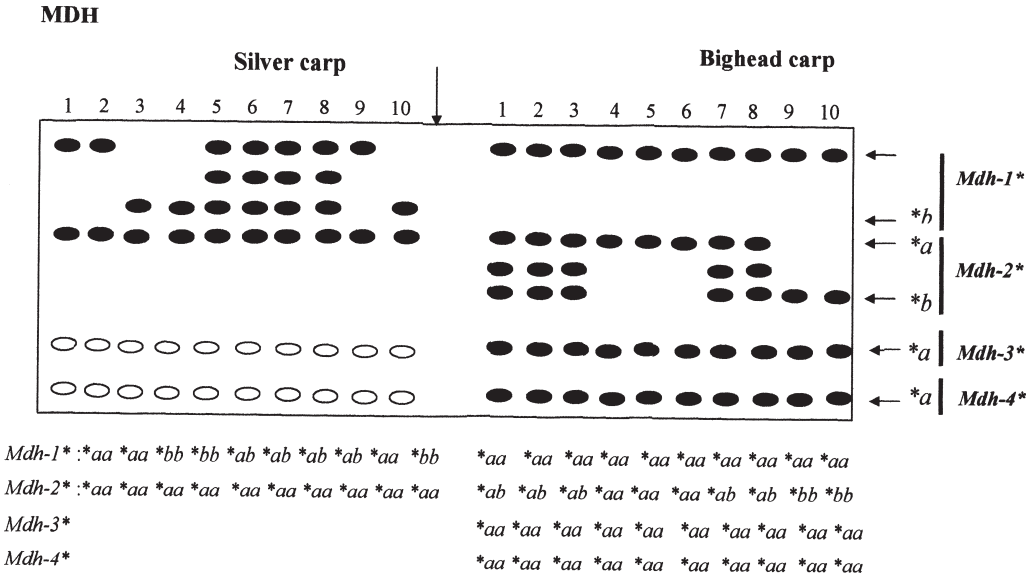

Fig 1. *Schematic representation of electrophoretic patterns of* Mdh-1*, Mdh-2*, Mdh-3* *and* Mdh-4* *in silver and bighead carps populations. Empty circles showing the absence of bands in the specific locus.*

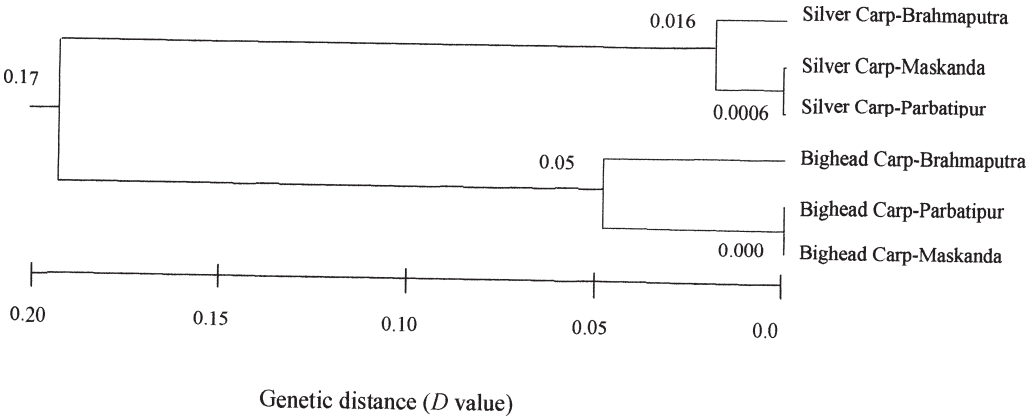

Fig 2. *UPGMA dendrogram showing genetic distance of silver and bighead carp collected from six hatcheries*

silver and bighead carps (Table 5). A dendrogram was drawn by the UPGMA method as shown in Fig. 2. The dendrogram showed that three silver carp populations were separated from the bighead carp populations by the *D* value of 0.17. Among the silver carp populations, Maskanda and Parbatipur formed a cluster and separated from the Brahmaputra population by the *D* value of 0.016. On the other hand, among the bighead carp populations Maskanda and Parbatipur formed a cluster and separated from the Brahmaputra by the *D* value of 0.05. These results indicated that very small numbers of brood stocks were used for hatchery propagation which may cause lower genetic distance

among populations. Also the same sources of broods may be used in both Parbatipur and Maskanda population whereas Brahmaputra stocks might be genetically isolated. According to Sattar and Das (2002), the silver and bighead carp founder stocks were introduced in Bangladesh from China; and secondly, introduction again from China and small number of stocks from Nepal which had poor or unknown genetic quality. The parent fish of these stocks performed low survival and breeding rate might lead to low genetic variation.

Since 1985 the artificial breeding of silver and bighead carps was started in Bangladesh and initially these carp seeds were distributed in four hatcheries (Sattar and Das, 2002). Under the national planning, the Chinese carps were recently introduced from China and mixed with their old existing stock. As a result, the *outcome generation* degraded and lost their genetic diversity. Due to rapid growth performance in pond aquaculture and livelihood demand of poor people, hatchery owners started to produce hybrid between silver and bighead carps. As a result, the original stock or genetic quality in the strain has been deteriorated. Mia *et al*. (2002) identified the hybrid using microsatellite DNA loci and suggested that the hybridization or introgression might be the reason for deterioration of quality. However, the present results based on allozyme marker revealed the genetic erosion of the existing silver and bighead carp stocks in Bangladesh. Because of the aquaculture potential of these two carps new stocks from their original sources should be transferred into Bangladesh.

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Refferences

Brummett, R., R.O. Smitherman and R.A. Dunham. 1988. Isozyme expression in bighead carp, silver carp and their reciprocal hybrids. *Aquaculture*, **70**: 21-28.

Clayton, J.W. and D.N. Tretiak 1972. Amine-citrate buffers for pH control in starch gel electrophoresis. *J. Fish. Res. Bd. Can*., **29**: 1169-1172.

Khan, M.M.R. 2002. Study of inbreeding depression of rohu (*Labeo rohita*) in both hatchery and wild populations of Bangladesh by allozyme electrophoresis, 66 pp. SUFER-DFID report.

Lewontin, R.C. and J.L. Hubby 1966. A molecular approach to the study of genetic heterozygosity in natural populations. ÀÀ. Amount of variation and degree of heterozygosity in natural populations of *Drosophila pseudoobscura*. *Genetics*, **54:** 595-609.

Li, S.F., B.Y. Zhou, C.K. Ni and Z.Q. Chen 1987. A genetic study of the growth performance of silver carp from the Chiangjiang and Zhujiang rivers. *Aquaculture*, **65**: 93-104.

Li, S.F., L.Z. Wu, J. Wang, Q.R. Chou and Y.L. Chen 1990. Comprehensive genetic study on Chinese Carps. Shanghai Scientific and Technical Publishers, Shanghai.

Mia, M.Y., J.B. Taggart, A.E. Gilmour, T.K. Das, M.A. Sattar, M.G. Hussain, M.A. Mazid, B.J. MacAndrew and D.J. Penman 2002. Development of DNA microsatellite loci in Chinese carps and application to detection of hybridization in broodstock. In: D.J. Penman, M.G. Hussain, B.J. McAndrew and M.A. Mazid (Eds.). *Proceedings of a workshop on Genetic Management and Improve ment Strategies for Exotic Carps in Bangladesh,* P-51-57.

Nei, M. 1972. Genetic distance between populations. *Am. Nat.*, **106**: 283-292.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, **89**: 583-590.

Penman D.J., M.G. Hussain, B.J. McAndrew and M.A. Mazid 2002 (a). *Genetic management and improvement of exotic carp species in Bangladesh*, 122 pp. Bangladesh Fisheries Research Institute.

Penman D.J., G.C. Mair, B.J. McAndrew, M.G. Hussain, Y. Basavaraju and P.A. Tuans 2002 b. *Aquaculture News*, April 2002. 19-22.

Roderick, D.M.P. 2000. TREEVIEW Tree drawing software for Apple Macintosh and Microsoft Windows. Tree View manual, 17 pp.

Sattar, M.A. and T.K. Das 2002. Broodstock management of Chinese carps and dissemination strategy at NFEP, Parbatipur. 43-50. In. D.J. Penman, M.G. Hussain, B.J. MacAndrew and M.A. Mazid (Eds.). *Proceedings of a workshop on Genetic Management and Improve ment Strategies for Exotic carps in Bangladesh.*

Shaklee, J.B., F.W. Allendorf, D.C. Morizot and G.S. Whitt 1990. Gene nomenclature for protein-coding loci in fish. *Trans. Am. Fish. Soc.*, **119**:2-15.

Shaw, C.R. and R. Prasad 1970. Starch gel electrophoresis of enzymes: A compilation of recipes. *Biochem. Genet.*, **4** (2): 297-320.

Siegismund, H.R. 1995. G-stat, version 3.1: Genetical statistical programs for the analysis of population data. Department of Plant Ecology, Botanical Institute, Oester Farimagsgade 2D, DK-1353 Copenhagen K, Denmark.

Yeh, F.C., R.C. Yang and T. Boyle 1999. POPGENE VERSION 1.31: Microsoft Window- based Freeware for Population Genetic Analysis. ftp://ftp.microsoft.com/Softlib/HPGL.EXE.

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