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Genetic Variation in Four Hatchery Populations of Thai Pangas, *Pangasius hypophthalmus* of Mymensingh Region in Bangladesh Using Allozyme Marker

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Abstract: To investigate genetic variation four hatchery populations (Shambhuganj, Brahmaputra, Anudan and Bhai-Bhai) of Thai pangas (*Pangasius hypophthalmus*) of Mymensingh region in Bangladesh were analyzed by allozyme electrophoresis. Using six enzymes, nine presumptive loci were identified where three (Est-1*, Gpi-2* and Pgm*) were polymorphic ($p \leq 0.95$) in Anudan and Brahmaputra populations and one locus, Est-1* was polymorphic in Shambhuganj population. No polymorphic locus was found in Bhai-Bhai population. The mean proportion of heterozygous loci per individual was 11.3±8.5% which ranged from 0.0% (Bhai-Bhai population) to 20% (Anudan population). The mean number of allele per locus was 1.3 that ranged from 1.0 (Bhai-Bhai population) to 1.6 (Anudan population). The UPGMA dendrogram showed that the four populations were divided into two groups by the genetic distance, D=0.18 but the populations were not geographically isolated. Finally the results indicated that the Anudan and Brahmaputra populations were showed higher genetic variation whereas Bhai-Bhai population lost their genetic variation completely.

Key words: Thai pangas (*Pangasius hypophthalmus*), hatchery populations, genetic variation, allozyme marker

INTRODUCTION

Pangasius hypophthalmus is locally called Thai pangas and the Ministry of Fisheries and Livestock (MOFL) initially introduced it into Bangladesh during 1990 from Thailand. Induced breeding of the species was started in 1993 in the hatcheries of Bangladesh. Then the artificial propagation has been practiced over the country and the popularity of the fish has been increased by the facts of its rapid growth, good taste and size. But the molecular characteristics were not into account for its sustainable production.

The large-scale production of fries of Thai pangas (*P. hypophthalmus*) is being carried out in private as well as in government sectors without considering genetical background and genetical stability. The fish hatchery owners don't recruit brood fish for breeding from original sources in a regular interval and they always try to use same broods and their offspring as broods stock year after year. So, inbreeding depression such as abnormality in growth and reduced fecundity, increase in disease resistance etc. occurred (Tarnchalanukit, 1986). So, the genetic status of Thai pangas in Bangladesh is needed to be analyzed using allozyme electrophoresis.

Pouyaud *et al.* (1998) and Roberts and Vidthayanon (1991) carried out allozyme electrophoresis studies of *Pangasius* genera. The allozyme studies of Thai pangas were not undertaken yet to identify the genetic variations in hatchery populations of Bangladesh.

Among the 13 exotic species Thai pangas is considered as a common food fish for the peoples of Bangladesh including poor livelihood peoples due to its availability, taste and cheap. Thai pangas is still in under pressure of inbreeding as a result quality seed production and brood stock management are faced difficulties that is why the study was carried out to clarify genetic variation of *P. hypophthalmus* using four hatchery populations from Mymensingh region in Bangladesh.

MATERIALS AND METHODS

Fish samples: The fingerlings of Thai pangas were collected from four different hatcheries i.e., Shambhuganj Government Fish Seed Multiplication Farm, Brahmaputra Fish Seed complex, Anudan hatchery and Bhai-Bhai Fish

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Table 1: Populations of Thai pangas used in the present study

Population No.	Population*1	No. of individuals	Date of collection
1	Shambhuganj Govt. Fish Seed Multiplication Farm (Sadar, Mymensingh) [Shambhuganj]	20	April 11, 2002
2	Brahmaputra Fish Seed Plant (Mymensingh) [Brahmaputra]	20	April 18, 2002
3	Anudan Hatchery (Charpuliamary, Mymensingh) [Anudan]*2	20	April 04, 2002
4	Bhai-Bhai Fish Seed Plant (Charpuliamary, Mymensingh) [Bhai-Bhai]*2	20	April 13, 2002

*1: Collection of samples with the district and Sadar area; Parenthesis showing the code name of each population
*2: These populations are very closely located and other hatchery is about 3-5km

Table 2: Ten enzymes were surveyed where six were examined and muscle tissues were showed better resolution for electrophoresis of Thai pangas. Two buffers*2 (CA 6.1 and CA7.0) used and CA 6.1 showed better performance

Types of Enzymes	Enzyme patterns	E.C. Number	Tissue*1
Esterase (EST)	Monomer	3.1.1. -	M\L
Glycerol-3-phosphate dehydrogenase (G3PDH)	Dimer	1.1.1.8	M
Glucose-6-phosphate isomerase (GPI)	Dimer	5.3.1.9	M\L
Lactate dehydrogenase (LDH)	Tetramer	1.1.1.27	M
Malate dehydrogenase (MDH)	Dimer	1.1.1.37	M
Phosphoglucomutase (PGM)	Monomer	5.4.2.2	M

*1 M: muscle; L: liver
*2CA 6.1: amine-citrate buffer (pH=6.1), CA 7.0: amine-citrate buffer (pH=7.0) (Clayton and Tretiak, 1972)

Seed Plant, Mymensingh during April, 2002. Details of the sampling localities, number of samples and collection date are given in Table 1. All the samples were reared in the newly constructed ponds after collection. Twenty individuals from each population were caught randomly after they get a size so that muscle and liver samples could be taken for allozyme work. The muscle and liver tissues were collected from each fish separately and immediately frozen at –20°C and stored at this temperature until electrophoretic analysis.

Allozyme electrophoresis: In the experiment, horizontal starch gel electrophoresis (Shaw and Prasad, 1970), using citric acid-aminopropylmorpholine buffer (pH 6.1) was used for allozyme work. The enzymes analyzed, abbreviation of enzymes, enzyme patterns, E.C. numbers, tissue types and buffer systems for horizontal starch-gel electrophoresis are shown in Table 2. After electrophoresis, the gel slices (about 1 mm thickness) were histochemically stained for different enzymes as described by Allendorf *et al.* (1977) and Aebersold *et al.* (1987) with some modifications.

Loci were numbered consecutively from the anodal to the cathodal side. Thus, the most anodal locus was designated '1'. Gene nomenclature followed by 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 analysis: Allele frequencies were calculated directly from observed genotypes. The distribution of observed genotypes was compared with that expected, calculated from the Hardy-Weinberg equilibrium using a χ^2 test.

When the most common (major) allele existed in a frequency less than or equal to 0.95 at a given locus, this locus was regarded as polymorphic. The mean proportions of heterozygous loci per individual, mean proportion of polymorphic loci per population, average number of alleles per population were calculated so as to show the extent of genetic variability (Lewontin and Hubby, 1966; and Lewontin, 1974). Expected (He) and observed average heterozygosity (Ho) were also calculated (Nei, 1972).

Coefficient of genetic variation (G_{ST}) was also calculated in order to estimate diversity between sample lots. Genetic distance values (D) (Nei, 1972) were calculated from allelic frequencies for all possible pairs of sample lots. The analyses of allozyme data were performed using G-Stat, version 3.1 (Siegismund, 1995) and TREEVIEW (Page, 2000). Based on the D-values, a dendrogram was made by the unweighted pair group method using arithmetic average (UPGMA) method (Nei, 1987).

RESULTS

Allele frequencies: The electrophoretic patterns of muscle samples showed that the genes at nine presumptive loci controlled the enzymes. Allele frequencies were calculated directly from observed genotypes at nine loci from four populations (Table 3). Esterase (EST) was exhibited three banded patterns consisted of one homodimers and two heterodimers, presumably controlled by 2 loci, Est-1*and Est-2*, where Est-2* was not readable due to complex banding pattern (Table 2). Three alleles *a, *b and *c were detected in anodal Est-1* (Fig. 1). Allele *c was rare and only found in Brahmaputra and Anudan populations with the allelic frequencies of 0.025 and 0.075, respectively. The glyceroldehyde-3 phosphate dehydrogenase (G3PDH) was dimeric enzyme and probably controlled by at least two different loci G3pdh-1* and G3pdh-2*. The band resolution of G3pdh-1* was faint in all the populations and it was difficult to interpret. The electrophoretic

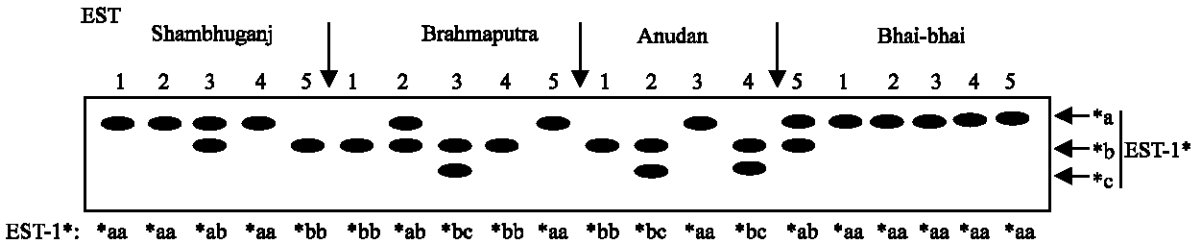

Fig. 1: Schematic representation of electrophoregram of esterase (EST) showing genetic variation among representative individuals (1-5) from Shambhuganj, Brahmaputra, Anudan and Bhai-Bhai populations of *P. hypophthalmus*. Symbol *a, *b and *c indicates three different alleles of the Est-1* locus. The respective genotypes are shown below in each lane for a particular individual

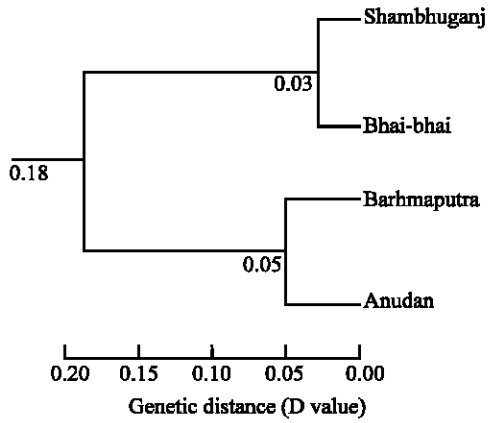

Fig. 2: The Dendrogram (UPGMA) showing genetic distance among four populations of Thai pangas

Table 3: Allele frequency at nine presumptive loci of Thai pangas samples

		Allele frequencies			
Locus	Allele	Shambhuganj	Brahmaputra	Anudan	Bhai-Bhai
Est-1*	*a	0.750	0.275	0.450	1.000
	*b	0.250	0.700	0.475	-
	*c	-	0.025	0.075	-
G3pdh-2*	*a	1.000	1.000	1.000	1.000
Gpi-1*	*a	1.000	1.000	0.975	1.000
	*b	-	-	0.025	-
Gpi-2*	*a	1.000	0.250	0.650	1.000
	*b	-	0.750	0.350	-
Ldh-1*	*a	1.000	1.000	1.000	1.000
Ldh-2*	*a	1.000	1.000	1.000	1.000
Mdh-1*	*a	0.975	1.000	1.000	1.000
	*b	0.025	-	-	-
Mdh-2*	*a	1.000	1.000	1.000	1.000
Pgm*	*a	1.000	0.575	0.625	1.000
	*b	-	0.425	0.375	-

patterns of G3pdh-2* showed one genotype by the single allele, *a. Glucose-6-phosphate isomerase (GPI) was dimeric enzyme and heterozygotes presumably controlled by two different loci, Gpi-1* and Gpi-2*. In case of Gpi-1* locus, two alleles, *a and *b were detected in all the population. Allele *b was rare and only found in Anudan hatchery with the allele frequency of 0.025. In case of Gpi-2* locus, allele *a was common in all populations except Brahmaputra population where the allele frequency of *b was 0.750. The lactate dehydrogenase (LDH) was tetrameric enzyme controlled by at least two loci Ldh-1* and Ldh-2* and showed monomorphic in all the populations. The malate dehydrogenase (MDH) was dimeric enzyme and controlled by two different loci, Mdh-1* and Mdh-2* whereas Mdh-1* had two alleles (*a and *b) and allele *b was found in Shambhuganj population. The phosphoglucomutase (PGM) was monomeric enzyme controlled by the single locus Pgm* and it showed three genotypes, *aa, *ab and *bb produced by two alleles, *a and *b. Major allele *a was observed at the frequencies between 1.00 and 0.575 in all the populations. In Shambhuganj and Bhai-Bhai populations, allele *b was not observed, whereas in other two populations, allele *b appeared at the frequencies between 0.425 and 0.375.

The chi-square test was made in all the cases of polymorphic loci between observed and expected genotypes, based on Hardy-Weinberg equilibrium. The test was not effective in the most cases in which the expected values were <5. However, Est-1* locus for Anudan and Brahmaputra populations showed significant result (Table 4).

Genetic variability: The mean proportion of heterozygous loci per individual was 11.3±8.5% (mean±SD) from the average number of 4 populations and ranged from 0.0% (Bhai-Bhai population) to 20% (Anudan population) (Table 5). The mean proportion of polymorphic loci per population was 19.4±16.7% on an average and ranged from 0.0% (Bhai-Bhai population) to 33.3% (Brahmaputra and Anudan populations). The mean number of allele per locus was 1.3±0.3 on an average and ranged from 1.0 (Bhai-Bhai population) to 1.6 (Anudan population). The

Table 4: Analyzed sample size and χ^2 test of fit to Hardy-Weinberg equilibrium

		Est-1*		G3pdh-2*		Gpi-1*		Gpi-2*		Ldh-1*		Ldh-2*		Mdh-1*		Mdh-2*		Pgm*	
Population	Sample size	χ^2	d.f.	χ^2	d.f	χ^2	d.f.	χ^2	d.f.	χ^2	d.f.	χ^2	d.f.	χ^2	d.f.	χ^2	d.f.	χ^2	d.f.
Shambhuganj	20	0.09	2	-	-	-	-	-	-	-	-	-	-	0.01	1	-	-	-	-
Brahmaputra	20	15.33*	3	-	-	-	-	0.80	2	-	-	-	-	-	-	-	-	1.61	2
Anudan	20	13.44*	3	-	-	0.01	1	2.32	2	-	-	-	-	-	-	-	-	1.28	2
Bhai-Bhai	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

* Agreement with the 5 or more expected frequency value

Table 5: Genetic variabilities at nine loci of Thai pangas samples. The mean proportion of polymorphic loci per population, mean proportion of heterozygous loci per individuals, mean no. of alleles per locus and heterozygosity ratio were higher in Anudan and Brahmaputra populations

	Variability					
				Heterozygosity		
Population	Mean proportion of polymorphic loci* per population (%)	Mean proportion of heterozygous loci per individual (%)	Mean no. of alleles	Ho	He	Ho/He
Shambhuganj	11.1	10.0	1.2	0.050	0.048	1.042
Brahmaputra	33.3	15.0	1.4	0.083	0.149	0.557
Anudan	33.3	20.0	1.6	0.106	0.177	0.599
Bhai-Bhai	0.0	0.0	1.0	0.0	0.0	0.0
Mean±SD	19.4±16.7	11.3±8.5	1.3±0.3	0.060±0.046	0.094±0.083	0.550±0.427

*$p \leq 0.95$

Table 6: Genetic identities (in above diagonal) and distances (in below diagonal) (Nei, 1972) among four populations of Thai pangas samples based on nine loci

Population	1	2	3	4
Shambhuganj	-	0.86	0.94	0.97
Brahmaputra	0.15	-	0.95	0.83
Anudan	0.07	0.05	-	0.92
Bhai-Bhai	0.03	0.18	0.09	-

observed heterozygosity (Ho) was 0.060±0.046 on an average and ranged from 0.000 (Bhai-Bhai population) to 0.106 (Anudan population). The average heterozygosity (He) was 0.094±0.083 and ranged from 0.000 (Bhai-Bhai population) to 0.177 (Anudan population).

Genetic differentiation: In the present study coefficient of gene differentiation (G_{ST}) (Nei, 1972) was estimated to be 0.792, showing high genetic differentiation among populations. A UPGMA dendrogram was also drawn (Fig. 2) where four populations were divided into two groups by the genetic distance, D=0.18 (Table 6). Group one was comprised with Shambhuganj and Bhai-Bhai populations and they were separated by the genetic distance D=0.03 whereas group two was comprised with Brahmaputra and Anudan populations and they were separated by the genetic distance D=0.05.

DISCUSSION

In Anudan and Brahmaputra populations three loci Est-1*, Gpi-2* and Pgm* were polymorphic whereas other two populations were monomorphic except the Est-1* locus of Shambhuganj population. In the Shambhuganj population the allele frequencies of *b at Est-1* locus was 0.250. Also rare allele (*b=0.025) were observed in Anudan population at the Gpi-1* locus whereas Shambhuganj population showed monomorphic (*a=1.00) (Table 3). So, the higher allelic variations were observed in Anudan population.

In the present study, the observed average proportion of polymorphic loci per population was 19.4%. This value is relatively similar to the reported average 22% in freshwater species like dace (Hanzawa *et al*., 1988) and 18% of 20 species of pangasiid catfish (Pouyaud *et al*., 1998) but much lower (65.22%) than yellow catfish *Mystus nemurus* (Leesa *et al*., 2000). Therefore, the studied *Pangasius* population was showed lower polymorphism than the above mentioned other catfishes. The average proportion of heterozygous loci per individual (11.3%) and the mean number of alleles per locus (1.3) obtained in this study were also lower than the average value of *Clarias macrocephalus*, which was reported to range between 1.2-1.4 (Na-Nakorn *et al*., 1998).

The average heterozygosity (Ho=0.060; He=0.094) estimated in the present study lower than the average value 0.091 of 20 species of pangasiid catfish, which was reported to range between 0.000-0.1826 (Pouyaud *et al*., 1998). The similar average heterozygosity (0.061) of *Clarias macrocephalus*, which was reported to range between 0.038-0.080 (Na-Nakorn *et al*., 1998). Thus, the Thai pangas is maintaining lower heterozygosity to the average of the Pangasiidae family. In addition, the Anudan and Brahmaputra populations were showed higher observed and expected heterozygosity (Ho=0.106 and 0.083; He=0.177 and 0.149 respectively) than the above-mentioned data. These results indicated that the Anudan and Brahmaputra hatchery samples were maintaining higher genetic variation.

The G_{ST} value (0.792) of the total population is equivalent to those estimated in other freshwater fishes

such as loach (0.774) (Khan and Arai, 2000) and freshwater gobi (0.698) (Shimizu et al., 1993). This is suggesting that a larger proportion of genetic variation in the Thai pangas, which may be due to difference among populations from different hatcheries.

Based on genetic distance (D-value), the four populations can be grouped into two as shown in the dendrogram (Fig. 2). First, group-1 was consisted with Shambhuganj and Bhai-Bhai populations at the D=0.18 than the group-2 populations which was constructed Anudan and Brahmaputra populations. (Table 6 and Fig. 2). Furthermore, in group-1, the Shambhuganj was separated from Bhai-Bhai population at D=0.03. In group-2, the Brahnmaputra population was differentiated from Anudan population at D=0.05. Pouyaud *et al.* (1998) reported that the average distances were found within the species *P. polyuranodon* (D=0.106) between population of Kalimantan and the population of Chao Phraya or *P. micronema* (D=0.145) between population of Teluk Kuantan in Sumatra and population of solo in Japan. Leesa *et al.* (2000) mentioned that the D-values of yellow catfish *Mystus nemurus* ranged from 0.005 to 0.164 and suggested that the highest genetic distance among them was the subspecies level. Similar results were observed by Shimizu *et al.* (1993) and also suggested that the highest genetic differentiation among the five groups of *Rhinogobius* was the species or subspecies level. On the other hand, the genetic distance (D=0.109) between interspecies of *P. nasutus* and *P. conchophilus* and also similar value (D=0.158) shown in the distance between *P. bocourti* and *P. djambal* (Pouyaud *et al.*, 1998). Nei found that in a variety of animals, D is approximately 1.0 for between species comparisons, around 0.1 for subspecies, and 0.01 for local races. Ayala (1975) reported that the D-value between subspecies is approximately 0.20. Considering from the above-mentioned criteria, the studied *P. hypophthalmus* may be fallen into the local race or population.

Among the studied four populations, the Anudan and Bhai-Bhai hatchery was very closely located (Table 1) but genetically Anudan was showed different. This may be due to different sources of brood.

Regarding the genetic information of *P. hypophthalmus*, it is able to know the genetic variability, which will be provided a clear concept on the conservation of genepool for sustainable production of this commercial friendly species year after year. Usually this constitutes a baseline in the construction of genetic structure of our native species *Pangasius pangasius*, which is going to be endangered (Sreenivasan, 1995) and revived this tasty species by undertaking a subsequent hybridization program between *P. pangasius* and *P. hypophthalmus*.

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