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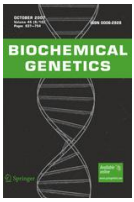

Population Genetic Structure of Endemic and Endangered Yellow Catfish, *Horabagrus brachysoma*, Using Allozyme Markers

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The allozyme variation and population genetic structure of *Horabagrus brachysoma in three natural populations from the southern part of the Western Ghats region, India, were investigated by polyacrylamide gel electrophoresis. Variations at 14 loci from 14 enzyme systems were analyzed. The allozyme analysis revealed a high level of genetic variation in this species, with an average of observed alleles per locus of 2.357 and observed heterozygosity of 0.178. The positive value of the fixation index* (F_{IS} = 0.507) implied a significant deficiency of heterozygosity at the population level. The highly significant probability ($P < 0.0001$) for the overall loci suggested that the three sample sets were not part of the same gene pool.

KEY WORDS: allozyme; polymorphism; heterozygosity; *Horabagrus brachysoma*; genetic variation.

INTRODUCTION

Horabagrus brachysoma is an endemic, cultivable yellow catfish belonging to the family Bagridae. It is found in rivers originating from the southern part

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of a biodiversity hotspot, the Western Ghats of South India (Myers et al. 2000). Once found in abundance, the species has recorded a sharp decline in catches due to overexploitation and is now restricted to a few rivers of Kerala and South Canara (the Nethravathi, Chalakkudy, and Meenachil rivers). A workshop of the Conservation Assessment Management Plan (CAMP) was held in 1997 to evaluate the status of freshwater species of India; it categorized this species as endangered based on the IUCN criteria (CAMP 1998). Captive breeding and milt cryopreservation techniques have been perfected in this species (Ponniah et al. 2000). No information is available, however, on polymorphic protein/allozyme patterns of this species across the range of its natural distribution.

Examination of genetic variation by electrophoresis of the primary gene products (proteins) provides a powerful tool for population discrimination and identification (Ferguson 1980). Information on stock structure is vital for planning management and conservation of natural resources, and it is useful in genetic improvement programs. Stock identification of several species has been carried out using these techniques (Shaklee et al. 1990; Ferguson et al. 1995; Lal et al. 2004; Salini et al. 2004). This paper is an initial attempt to estimate the degree of genetic variation at protein-coding loci detected by electrophoresis in *H. brachysoma* from three riverine systems along the Western Ghat region of India and to determine their population structures.

MATERIALS AND METHODS

Specimens of *H. brachysoma* were obtained through commercial catches from three rivers, the Meenachil, Chalakkudy, and Nethravathi. Only one sample site was chosen for each population because these rivers are short in length (average 76 km), and they do not have tributaries. The Meenachil site was at Kumarakom (09°33′ N, 76°25′ E), Chalakkudy at Kanakkankadavu (10°08'N, 76°07'E), and Nethravathi at Kankanadi (12°52′ N, 74°54′ E) (Fig. 1). A total of 210 specimens were collected for the genetic variability study (70 from each of the three rivers). The liver tissues were collected at the site itself and immediately frozen in liquid nitrogen (−196°C). The samples were transported to the laboratory and stored at −80°C until analysis.

Each frozen liver sample (approximately 100 mg) was homogenized in 250 mg/ml extraction buffer (0.17 M sucrose, 0.2 M EDTA, 0.2 M Tris-HCl, pH 7.0). The homogenized samples were centrifuged for an hour at 12,000 rpm at 4°C, and the supernatant was recentrifuged for 45 min. Enzyme systems were assessed using the middle portion of the supernatant in a vertical polyacrylamide gel (7.25%) electrophoresis (Amersham Biosciences). Electrophoresis was carried out using TBE buffer system

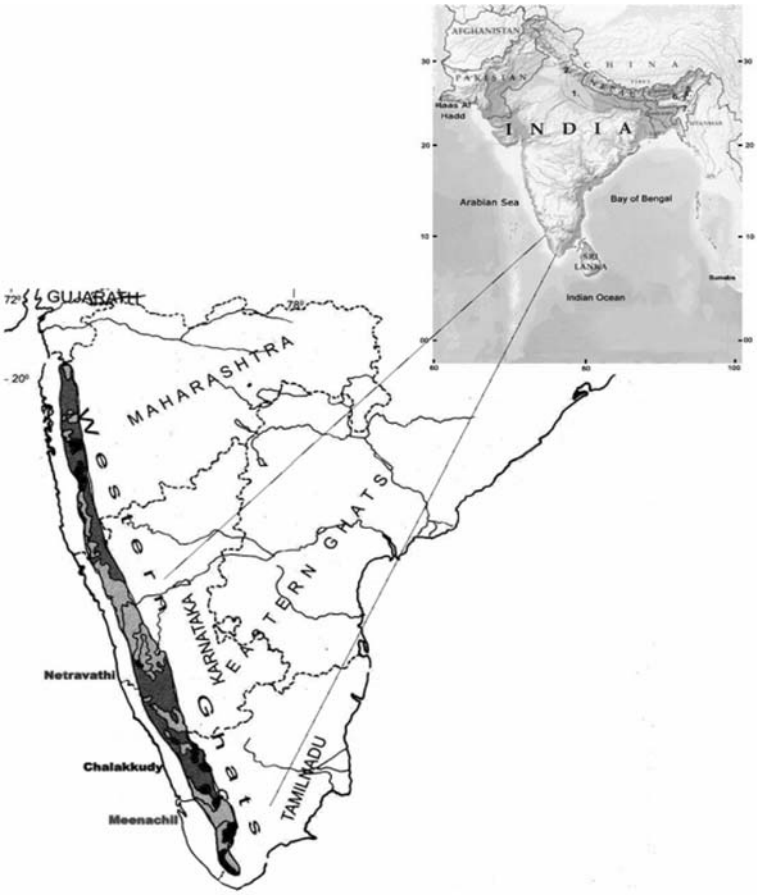

Fig. 1. Sample collection sites for *Horabagrus brachysoma* used in this study

(90 mM Tris-borate, 2 mM EDTA, pH 8.0) at a constant 150 V at 4°C, except for SOD, which was resolved in TG buffer system (5 mM Tris-Cl and 0.038 M glycine, pH 8.3). In all, 25 enzyme systems were examined (Table 1). The alleles of an enzyme were visualized by histochemical staining following the procedures outlined by Shaw and Prasad (1970). The gels were documented using an Imagemaster 1D gel documentation system (Amersham Biosciences).

The nomenclature of the loci and alleles was as recommended by Shaklee et al. (1990). For all the loci, the most common allele was assigned "100." Alternate alleles were designated as per their mobility, in relation to the most common allele. The parameters of genetic variation, such as

Table 1. Allozyme analysis in *Horabagrus brachysoma*

Enzyme	No. of loci	Locus	Alleles (EC no.)	Monomorphic/ polymorphic
Acid phosphatase	ns	*ACP**	*ns*	ns
Adenylate kinase	ns	*AK**	*ns*	ns
Alcohol dehydrogenase	ns	*ADH**	*ns*	ns
Alkaline phosphate	ns	*ALP**	*ns*	ns
Aspartate amino transferase	2	*AAT-1**	*100*	Monomorphic
		*AAT-2**	*100,117,126*	Polymorphic
Creatine kinase	ns	*CK**	*ns*	ns
Esterase	5	*EST-1**	*083,100*	Polymorphic
		*EST-2**	*100,106*	Polymorphic
		*EST-3**	*095,100*	Polymorphic
		*EST-4**	*100*	Monomorphic
		*EST-5**	*100*	Monomorphic
Fumerase	ns	*FUM**	*ns*	ns
Glutamate dehydrogenase	ns	*GDH**	*ns*	ns
Glucose dehydrogenase	1	*GLDH**	*080,089,100,117*	Polymorphic
Glucose phosphate isomerase	2	*GPI-1**	*100*	Monomorphic
		*GP1-2**	*096,100*	Polymorphic
Glucose-6-phosphate dehydrogenase	1	G_6PDH*	*086,100*	Polymorphic
α-Glycerophosphate dehydrogenase	1	αG_3PDH*	*088,100*	Polymorphic
Glyceraldehyde-3-phosphate dehydrogenase	2	*GAPDH -1**	*100*	Monomorphic
		*GAPDH -2**	*100*	Monomorphic
Hexokinase	ns	*HK**	*ns*	ns
Isocitrate dehydrogenase	ns	*ICDH**	*ns*	ns
Lactate dehydrogenase	2	*LDH-1**	*100*	Monomorphic
		*LDH-2**	*100,112,134*	Polymorphic
Malate dehydrogenase	1	*MDH**	*086,100*	Polymorphic
Malic enzyme	1	*ME**	*100*	Monomorphic
Octonol dehydrogenase	3	*ODH-1**	*100*	Monomorphic
		*ODH-2**	*091,100*	Polymorphic
		*ODH-3**	*100*	Monomorphic
Phosphogluconate dehydrogenase	ns	*6PGDH**	*ns*	ns
Phosphogluco mutase	1	*PGM**	*093,100*	Polymorphic
Pyruvate kinase	ns	*PK**	*ns*	ns
Superoxide dismutase	1	*SOD**	*093,100*	Polymorphic
Xanthine dehydrogenase	2	*XDH-1**	*093,100*	Polymorphic
		*XDH-2**	*100*	Monomorphic

Note: ns, Indicates that the enzyme did not yield any scorable activity

proportion of polymorphic loci ($P_{0.95}$ and $P_{0.99}$ criteria), heterozygosity at individual locus, and mean over all loci for each population, were calculated using the software Genetix version 4.05 (Belkhir et al. 1997). GenePop version 3.4 (Raymond and Rousset 1998) was used to assess conformity of the phenotypic frequencies to those expected under Hardy-Weinberg equilibrium. Exact *P*-tests for conformity to Hardy-Weinberg (probability and score test) were performed by the Markov chain method using GenePop

version 3.4. The phylogenetic relationships based on the genetic distance values generated from allozyme data among the three populations of *H. brachysoma* were determined through a dendrogram plotted following the unweighted pair group method with arithmetic averages (UPGMA; Sneath and Sokal 1973), using PopGene version 1.31 (Yeh et al. 1999).

RESULTS AND DISCUSSION

Fourteen out of 25 enzymes were found to give scorable activity that provided 25 loci. Of the 25 presumptive loci analyzed, 14 (56%) were found to be polymorphic (Table 1). A total of 45 alleles were detected among the 25 loci. Zymograms of esterase and glucose dehydrogenase are shown in Figs. 2 and 3, respectively. The mean number of observed (n_a) and effective (n_e) alleles per locus for overall populations was 2.357 and 1.829, respectively (Table 2). The genetic diversity expressed in terms of mean n_a is usually higher in species with wider geographic range, higher fecundity, greater longevity, and larger population size (Nevo et al. 1984). The mean value of n_a in *H. brachysoma* (2.357) exceeded that of many freshwater species, such as *Tenualosa ilisha* (1.49; Lal et al. 2004) and *Cirrhinus mrigala* (1.31; Singh et al. 2004). Slightly lower values were reported in other catfish species, such as *Clarias gariepinus*, *C. anguillaris*, and *C. albopunctatus* (Rognon et al. 1998), and in pangasiid catfishes (Pouyaud et al. 2000).

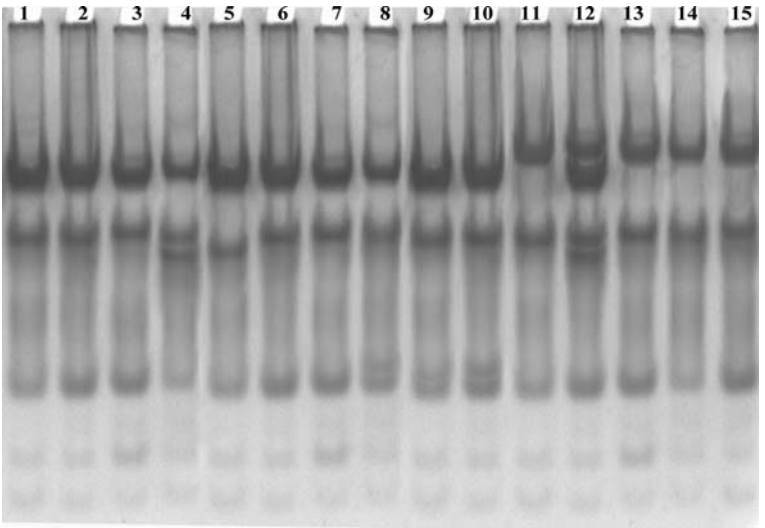

Fig. 2. Esterase pattern in *Horabagrus brachysoma*. Lanes 1–5, Meenachil. Lanes 6–10, Chalakkudy. Lanes 11–15, Nethravathi

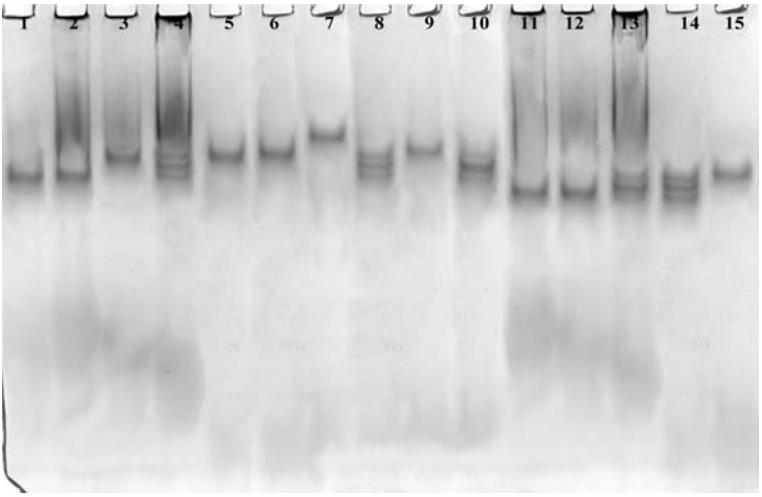

Fig. 3. Glucose dehydrogenase pattern in *Horabagrus brachysoma*. Lanes 1–5, Meenachil. Lanes 6–10, Chalakkudy. Lanes 11–15, Nethravathi

Observed heterozygosity (H_o) and expected heterozygosity (H_e) at all 14 polymorphic loci were 0.178 and 0.428, respectively (Table 2). H_o fell within the range of the other catfish *Clarias gariepinus*, *C. anguillaris*, *C. albopunctatus*, and *Heterobranchus longifilis* (Rognon et al. 1998) and that of many *Pangasius* species (Pouyaud et al. 2000). H_o obtained in the present study in *H. brachysoma* was lower than the H_e, indicating a deficiency of heterozygotes, except for one or two loci in each population. The deficiency of heterozygotes and deviations from Hardy-Weinberg in yellow catfish could be due to inbreeding, a situation caused by overexploitation leading to a decline of the species in the wild.

The probability test provided the evidence that the observed phenotypic frequencies for most of the loci deviated significantly ($P < 0.05$) from those expected under Hardy-Weinberg in all three populations. The F_{IS} values for

Table 2. Genetic variations within and among three populations of *Horabagrus brachysoma*

	Mean number of alleles		% of polymorphic loci		Mean heterozygosity	
Population	Observed	Expected	$P_{0.95}$	$P_{0.99}$	Observed	Expected
Meenachil	1.857	1.597	48	48	0.172	0.346
Chalakkudy	2.071	1.710	52	52	0.191	0.397
Nethravathi	2.071	1.615	52	52	0.170	0.347
Across populations	2.357	1.829	56	56	0.178	0.428

each locus ranged from -0.228 for *EST-3** to 0.885 for *LDH-2**, with an average of 0.507. For most of the loci the value of F_{IS} deviated significantly from zero, indicating a deficiency of heterozygotes, except in *EST-3**. Generally, where the loci did not conform to Hardy-Weinberg expectations, a significant lack of heterozygotes was observed as evidenced by the positive F_{IS} values. The significant values for the inbreeding coefficient (F_{IS}) for each locus in each population and in the overall population are given in Table 3.

The mean value of the coefficient of genetic differentiation (F_{ST}) was 0.154, and the average number of migrants per generation (N_m) was 1.376 (Table 3). This indicated that 15.4% of the total genetic differentiation occurred in the population of *H. brachysoma*. Similar values for F_{ST} were reported in populations of *Clarias anguillaris* ($F_{ST} = 0.15$) by Rognon et al. (1998). The same authors have reported a lower F_{ST} value (0.044) for populations of *Clarias gariepinus*. The genetic relatedness of the *H. brachysoma* populations derived using pairwise F_{ST} between populations differed significantly ($P < 0.0001$) from zero for all pairs of riverine locations, indicating significant heterogeneity between populations. In the present study, the overall and pairwise F_{ST} values fell within the range reported for freshwater fishes. The N_m value of 1.376 indicated chances of restricted migration between populations, and $N_m > 4$ suggested that gene flow between populations was adequate to counteract the effects of genetic drift in local populations (Kang and Chung 1997).

The genetic distance values (Nei 1978) ranged from 0.0299 to 0.0927 (Fig. 4), close to the average obtained by Shaklee et al. (1982) for

Table 3. F-statistics and gene flow for overall populations

Locus	Sample size	F_{IS}	F_{ST}	N_m
*AAT-2**	210	0.401	0.071	3.245
*EST-1**	210	0.676	0.781	0.070
*EST-2**	210	0.543	0.045	5.274
*EST-3**	210	−0.228	0.132	1.644
*G_3PDH**	210	0.361	0.081	2.838
*G_6PDH**	210	0.533	0.123	1.787
*GLDH**	210	0.340	0.281	0.640
*GPI-2**	210	0.744	0.007	34.125
*LDH-2**	210	0.885	0.072	3.233
*MDH**	210	0.725	0.022	11.184
*ODH-2**	210	0.518	0.207	0.958
*PGM**	210	0.322	0.004	58.835
*SOD**	210	0.426	0.204	0.977
*XDH-1**	210	0.606	0.025	9.906
Mean	210	0.507	0.154	1.376

Note: F_{IS}, inbreeding coefficient; F^{ST}, coefficient of genetic differentiation; N_m, average number of migrants per generation

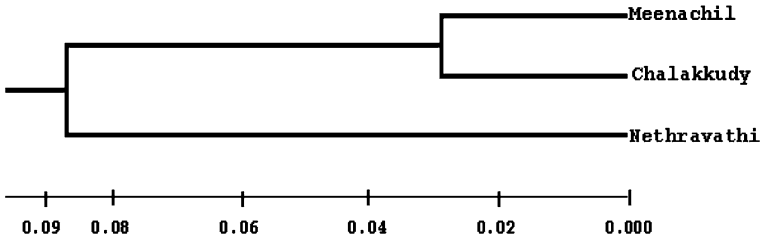

Fig. 4. UPGMA dendrogram based on Nei's (1978) genetic distance, showing the relationship among *Horabagrus brachysoma* populations

conspecific populations of marine and freshwater fish ($D = 0.05$, $I = 0.977$). In clariid catfishes, the genetic distance at the intraspecific level ranged from 0.008 to 0.29 in *Clarias gariepinus* and 0.005 to 0.043 in *C. anguillaris* (Rognon et al. 1998).

In conclusion, the allozyme studies alone provide positive proof for the existence of genetically different stocks of *H. brachysoma* in the three rivers along the Western Ghats. This occurrence of distinct stocks of yellow catfish could be interpreted in two ways: (1) lack of gene flow between populations as a result of geographic isolation so that forces such as random genetic drift had operated to cause genetic divergence and (2) local genetic adaptations to different environmental conditions.

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