Asian Fisheries Science **16** (2003): 229-234
Asian Fisheries Society, Manila, Philippines

Genetic Make Up of Exotic Catfish *Clarias gariepinus* in India

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Abstract

Twelve enzymes were assayed in *Clarias gariepinus* collected from India and Thailand. Genetic variation was observed at eight loci *viz. EST-1** and *2**, *AAT**, *ODH**, *MDH** *GPI** L, *GPI*-1*M and *HK**. Observed heterozygosities were found to be 0.149 and 0.152, and percent polymorphism 50% and 31.25% respectively in samples from India and Thailand. The results indicate that *C. gariepinus* in India could be the mix of different stocks belonging to different genetic lineages.

Introduction

Clarias gariepinus (Burchell, 1822) belonging to family Claridae is the native species of Africa. The species has drawn attention of aquaculturists because of its biological attributes that include faster growth rate, resistance to diseases and possibility of high stocking density. It has been introduced in several countries of Europe and Asia. Aquaculture of *C. gariepinus* is practiced in twenty countries with the total production of 3703 mt., out of which 94.7% (3505 mt) is reported from four countries, the bulk (2600 mt) of which is coming only from the Netherlands (FAO, 2000). It indicates that the culture prospects could not match the widespread introduction done for this species, which can be attributed to the certain negative impacts of *C.*

gariepinus. Due to high predatory nature together with omni voracity and prolificacy, C. *gariepinus* tends to impose a great threat to native fish fauna. Concern has also been raised against the possible introgression with native *Clarias* species in Asia, like *C. batrachus* and *C. macrocephalus* (Na-Nakorn et al. 1998). Such introgressions may not necessarily be the outcome of natural breeding, rather it can occur due to escape of farmed hybrids. Farmed production of hybrid (*C. gariepinus* x *C. macrocephalus*) in Thailand is estimated to be 71210 mt in the year 2002 (FAO, 2000).

Presence of *C. gariepinus* in Indian fish markets is known since 1993. It is hypothesized to have been brought in from Thailand through Bangladesh (Thakur, 1996). The legal norms were not followed during the introduction and on account of biodiversity concerns; the Government of India banned its culture. Consequently, the culture is not popular in the present scenario. The species with the unknown genetic make up exists posing threat to native fish fauna especially introgression of native *C. batrachus* population. The present study assesses the pattern of genetic variability in *C. gariepinus* available in India and compares with the reference samples of the same species from Thailand.

Materials and Methods

The tissue samples for *C. gariepinus* (n=42) from local fish market (Lucknow, India) were collected. The reference samples (n=7) were collected from Department of Aquaculture, Faculty of Fisheries, Kasetsart University, Bangkok Thailand in the year 2000. The fish were sacrificed to obtain liver and muscle samples that were stored at –80ºC till analysis. The tissue was mildly homogenized in chilled extraction buffer (0.17M Sucrose, 0.2M EDTA, 0.2M Tris- HCl, pH 7.0 for liver and 10% sucrose for muscle). Extraction of liver was done at 250mg.ml^{-1} and muscle at 125mg.ml^{-1}. Suspensions were centrifuged at 10,000 rpm at 4°C for an hour. Supernatant was again centrifuged for 20 minutes. Samples were run in the vertical electrophoresis units (gel size 8x7 cm, Hoeffer Scientific Mighty Small SE 250) through 7% polyacrylamide gels (Gopalakrishnan et. al. 1997) and Tris – Borate (500mM Tris, 650mM Boric Acid, 16mM EDTA pH 8.0) as running buffer. Electrophoresis was conducted at constant voltage of 150 V. The allozyme profiles were visualized using histochemical staining methods (Morizot and Schmidt, 1990). The nomenclature for loci and alleles was followed as per recommendations of Shaklee et. al. (1990). Most common allele in *C. gariepinus* (India) was designated as 100. Alternate alleles were designated as per their mobility relative to the most common allele. For the enzymes where liver and muscle expressed different loci, distinction was made by suffixing 'L' and 'M' respectively, with the name of the locus.

The data was analyzed using the software Genetix 4.0 (Belkir et. al., 1998) for estimates of classical variables of polymorphism, heterozygosity and inbreeding coefficient (F_{is}). Genepop version 3.3 (Raymond and Rousset, 1995) was used for performing probability test for Hardy Weinberg equilib-

rium, linkage disequilibrium and estimating genotypic and allelic heterogeneity.

Results and Discussion

The enzyme systems, E. C. number, locus designation and tissue studied are given in table 1. Twelve enzyme systems were analyzed that yielded sixteen loci exhibiting scorable banding patterns. Out of these, eight loci were polymorphic (P< 0.99). Allele frequencies are presented in table 2. Loci *EST-1* and -2*, AAT*, ODH*, MDH** were polymorphic in both the samples from India and Thailand, while loci *GPI** L, *GPI-* 1*M and *HK** displayed alternate alleles only in Indian samples. Parameters of genetic variation are

Table 1. Enzymes, loci scored to examine genetic structure in *Clarias gariepinus*

Enzyme	E.C. Number	Locus	Tissue
Aspartate amino transferase	2.6.1.1	*AAT**	liver
Esterase	3.1.1.-	*EST-1*, EST-2**	liver
Glucose phosphate isomerase	5.3.1.9	*GPI** L,	liver
		*GPI-1**M, *GPI-2**M	muscle
Glutamate dehydrogenase	1.4.1.3	*GLUD**	liver
α-glycerophosphate dehydrognase	1.1.1.8	*GPD**	liver
Malate dehydrogenase	1.1.1.37	*MDH**	liver
Phosphogluconate dehydrogenase	1.1.1.44	*PGDH**	liver
Octanol dehydrogenase	1.1.1.73	*ODH**	liver
Hexokinase	2.7.1.1	*HK**	liver
Xanthine dehydragenase	1.2.1.37	*XDH**	liver
Phospho glucomutase	2.7.5.1	*PGM-1**M, *PGM-2**M	muscle
Adenylate kinase	2.7.4.3	*AK**	liver

Table. 2. Allele frequencies for eight polymorphic allozyme loci in *Clarias gariepinus*

Locus	Alleles	*C. gariepinus* (India)	*C. gariepinus* (Thailand)
	(*n*)	42	7
*EST-1**	100	0.452	0.500
	106	0.357	0.286
	115	0.191	0.214
*EST-2**	100	0.486	0.357
	106	0.271	0.643
	110	0.243	0.000
*GPI**L	100	0.515	0.000
	130	0.485	1.000
GPI-1*M	*55*	0.054	0.000
	100	0.946	1.000
*HK**	94	0.037	0.000
	100	0.963	1.000
*AAT**	100	0.964	0.929
	131	0.036	0.072
*ODH**	100	0.988	0.786
	110	0.012	0.214
*MDH**	100	0.611	0.500
	113	0.167	0.000
	128	0.222	0.500

given in the table 3. The mean number of alleles per locus was 1.688 in Indian samples, while 1.375 in Thailand samples. The observed heterozygosities were comparable in Indian (0.149) and Thailand (0.152) samples. The values of observed heterozygosity (H_{obs}) and polymorphism (P) conform to the range previously reported for *C. gariepinus* (Agnese et. al., 1997; Vander Bank et. al., 1992.). Agnese et. al. (1997) found P to be 44 % and H_{obs} 0.152 in *C. gariepinus* from Senegal. Vander Bank et al. (1992) reported that H_{obs} values were 0.047 in wild population while 0.033 and 0.076 in two domesticated populations of *C. gariepinus* from South Africa. It is evident that C. *gariepinus* studied from different regions including wild and the introduced exhibits a wide range of variations in heterozygosity values. In present study, polymorphic loci (50%) in Indian samples at 99% criteria is high as compared to Thailand (31.25%) samples due to the presence of alleles in low frequency in Indian samples but lacking in Thailand samples. However, absence of these alleles viz. *EST- 2* 110, GPI-1** M *55, HK* 94, MDH* 113* in Thailand samples did not contribute significantly (P > 0.05) to observed genotypic heterogeneity. The present study reveals significant differences in genotypic proportions at *GPI** L and *ODH** loci between *C. gariepinus* samples from India and Thailand. The most common allele *GPI**L *100*, in Indian samples is conspicuously lacking in samples from Thailand. It is evident from the data that the samples of *C. gariepinus* collected from India and Thailand does not reflect concordant genetic make up.

Interestingly, the allele frequencies at two loci viz. *EST-1**, and *MDH** in *C. gariepinus* from India do not conform to the proportions expected under Hardy-Weinberg (HW) equilibrium (Table 4). Inbreeding coefficient (F_{is}) values significantly (P<0.05) deviated from zero at the two loci, pointing out deficiency (*MDH**) and excess (*EST-1**) of heterozygotes. Significant

Table 3. Genetic variation in *C. gariepinus* at 16 loci

	India	Thailand
Expected heterozygosity ($H_{exp.}$)	0.161	0.128
Observed heterozygosity (H_{obs})	0.149	0.152
Polymorphism (P<0.95)	31.25	31.25
(P<0.99)	50.00	31.25
Mean no of alleles / locus	1.688	1.375

Table 4. Heterozygosity and inbreeding coefficient (F_{is}) values for different allozyme loci in *Clarias gariepinus*

	India			Thailand		
	Hobs.	Hexp.	Fis	Hobs.	Hexp.	Fis
*EST-1**	0.905	0.632	–0.423*	1.000	0.622	-0.556
*EST-2**	0.457	0.631	0.289	0.143	0.459	0.727
*GPI**L	0.441	0.500	0.132	0.000	0.000	0.000
*GPI-1**M	0.107	0.101	–0.038	0.000	0.000	0.000
*HK**	0.074	0.071	-0.020	0.000	0.000	0.000
*AAT**	0.071	0.069	-0.025	0.143	0.133	0.000
*ODH**	0.024	0.024	0.000	0.429	0.337	-0.200
*MDH**	0.306	0.549	0.455*	0.714	0.500	-0.364
Multilocus	0.149	0.161	0.196	0.152	0.128	0.000

*locus does not conform to Hardy Weinberg proportions (P<0.05).
-ve and +ve F_{is} values indicate excess and deficit of heterozygotes

linkage disequilibrium (P<0.05) between *EST-1** and *MDH** was also noted in the samples from India. The observed parameters indicate that allele frequencies at these loci can not be said to be stable from one generation to the next. This clearly indicates the violation of assumptions of random mating and Mendelian inheritance in *C. gariepinus* samples from India. Departures from HW equilibrium can occur due to mixing of heterogeneous gene pools (Ferguson et al. 1995). Since the *C. gariepinus* is not found in Asia naturally, the different gene pools may not be different natural stocks. The introductions can take place from the populations present in different commercial hatcheries and farms that maintain reproductive isolation, consequently acquire altered allele frequencies due to founder effect or drift. It is likely that the genetic make up of *C. gariepinus*, thriving in India, is the outcome of mixing of stocks introduced from multiple sources having different genetic base.

Acknowledgements

The authors thank Dr. D. Kapoor, Director, NBFGR, Lucknow for the encouragement and guidance. We thank Prof. Uthairat Na- Nakoran, Department of Aquaculture, Faculty of Fisheries, Kasetsart University, Bangkok, Thailand, for providing the reference samples. The excellent assistance provided by Mr. R.S. Sah, Mr. B.K. Rao, Mr. Akhilesh Mishra, Mr. Rajesh Kumar and Mr. Sree Ram of NBFGR Lucknow, during sampling as well as analysis of samples are duly acknowledged.

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Manuscript received 16 January 2003 ; Accepted 29 May 2003