Genetic Divergence between Two Marine Catfish of Family Ariidae - *Arius maculatus* and *Osteogeneiosus militaris*

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ABSTRACT : Two species of marine catfish, *Arius maculatus* and *Osteogeneiosus militaris*, belonging to family Ariidae were analysed electrophoretically for genetic variation in 6 enzymes, alcohol dehydrogenase (ADH), malate dehydrogenase (MDH), lactate dehydrogenase (LDH), glucose dehydrogenase (GDH), malic enzyme (ME) and superoxide dismutase (SOD). Eighteen individuals of each species were studied. Two loci MDH and ADH were polymorphic in both. Average heterozygosity in *A. maculatus* was 1.46, while it was 2.5 in *O. militaris*. The allele frequencies were used to estimate Nei's genetic distance (D). The D value was calculated to be 0.6879. Two isozyme loci, ME and SOD, were found to be the most reliable species specific markers. No tissue specific loci were observed for the enzymes studied, the bands being identical in each case. The genetic distance observed between *O. militaris* and *A. maculatus* in this study suggests that they would be more appropriately classified as species of the same genus rather than being assigned separate genera. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 9 : 1188-1191)***

Key Words : Allozyme Polymorphism, *Arius maculatus*, *Osteogeneiosus militaris*

INTRODUCTION

Allozymes profiles yield useful information about genetic divergence among species and have been used to distinguish between the closely related species (Rognon et al., 1998; Mo et al., 2003). The allozyme variation is known to be genetically controlled and codominant and only slightly affected by environmental disturbances (McAndrew and Majumdar, 1983). Cumulative comparisons among loci between taxonomic groups can be summarised into indices of genetic similarity or distance (Menezes and Taniguchi, 1988), genetic distance being a measure of biochemical diversity in terms of the number of allelic substitutions per locus that have occurred during separate evolution of two populations or species.

A large number of catfish species constitute the commercial catfish fishery along the west and east coasts of India, however, the study of their biology has not received adequate attention (Bal and Rao, 1990). Their nutritive value is comparable to some of the quality fishes and they have increasing commercial potential. The catfish family Ariidae includes two commercially important genera, *Arius* and *Osteogeneiosus*, the latter being distinguishable only by the ossification of maxillary barbels. The present study uses the allozyme profiles to investigate the taxonomic relationship between these two genera.

MATERIALS AND METHODS

Experimental animal

Eighteen specimens each of *O. militaris* and *A. maculatus* were collected by trawling off Versova, Mumbai. The total length of the specimens analysed ranged between 7-8 cm in *A. maculatus* and 20-22 cm in *O. militaris*. They were kept frozen at -20°C prior to analysis.

Preparation of extract and PAGE

Skeletal muscle, liver and heart tissues were collected from individual specimens. The tissue was homogenised in 10 mM Tris/1 mM EDTA buffer, pH 6.8, and directly subjected to native polyacrylamide gel electrophoresis (PAGE) for phenotypic analysis. PAGE was done as described by Davis (1964). Separating gel of 7.5 and 4% stacking gel was used. Isozyme profiles were developed for ADH, LDH, MDH, GDH, SOD and ME, using the staining procedures given by Pasteur et al. (1988). The most common allele was designated 100 and the other alleles were labelled according to their migration speeds in relation to allele 100. R_d values of the bands were also calculated by dividing the distance migrated by the band with the distance migrated by bromophenol blue.

Analysis of data

The identity or difference of alleles at the same locus was decided by the position of isozymes on the same gel. Genetic identity or distance was calculated from the formula proposed by Nei (1972). Average heterozygosity was determined by totalling the number of observed heterozygotes for each locus, dividing this by the total number of individuals in the sample and then averaging over all loci (Hoelzel and Bancroft, 1988). Proportion of polymorphic loci was calculated by dividing the number of polymorphic loci observed by the total number of loci studied.

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Table 1. Allele frequencies at six loci in two marine catfishes

Locus	R_d value of the bands observed	Alleles observed	Species	
			O. militaris	*A. maculatus*
MDH	0.239	100	0.5	0.4375
	0.302	125	0.5	0.4375
	0.314	130	0	0.125
		x^2	0.312	8.4
		df	1	3
		n	18	18
ADH	0.222	100	0.5	0.75
	0.246	110	0.5	0
	0.269	120	0	0.25
		x^2	5.56*	2.3
		df	1	1
		n	18	18
LDH	0.255	100	1	1
		n	18	18
GDH	0.157	100	1	1
		n	18	18
SOD	0.619	100	0	1
	0.838	135	1	0
		n	18	18
ME	0.239	100	1	0
	0.275	115	0	1
		n	18	18

x^2: Difference between observed and expected phenotypic distributions.
n: Number of individuals.
* Not in Hardy Weinberg equilibrium ($p>0.05$).

Table 2. Genetic variability observed in two marine catfish species

	Genetic variability	
	O. militaris	*A. maculatus*
No. of loci observed	6	6
No. of polymorphic loci	2	2
Proportion of polymorphic loci	0.33	0.33
Mean no. of alleles	1.33	1.5
Average heterozygosity	2.5	1.46

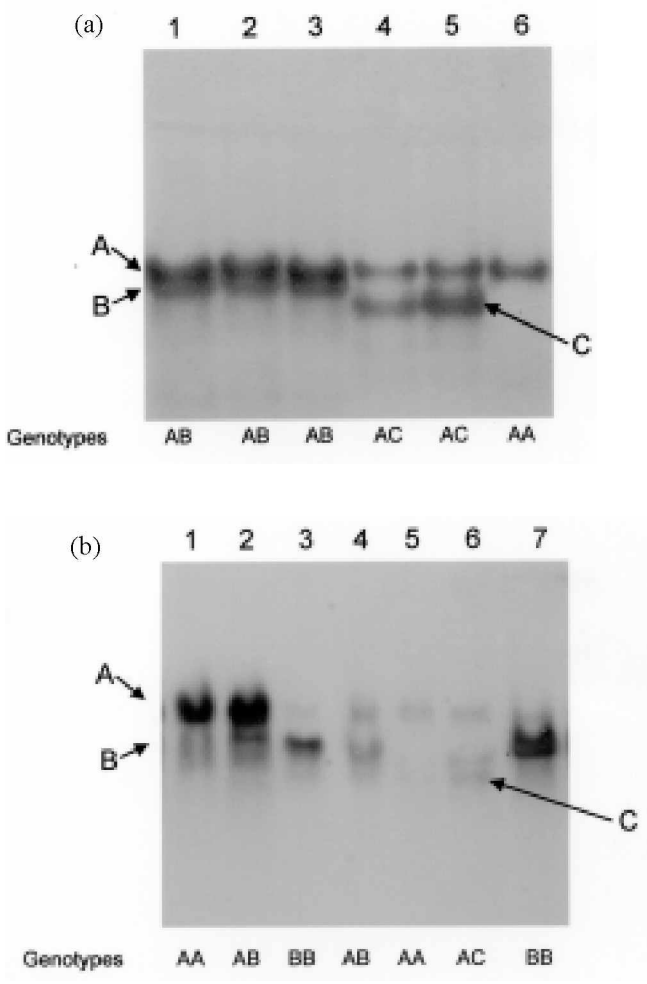

Figure 1. (a) ADH profile in muscle tissue of *O. militaris* and *A. maculates* and genotypes. Lanes 1-3: Individuals of *O. militaris*; lanes: 4-7: Individuals of *A. maculates*. Allele description: A is 100, B is 110, C is 120. (b) MDH profile in muscle tissue of *O. militaris* and *A. maculates* and genotypes. Lanes 1-4: Individuals of *O. militaris*; lanes: 5-10: Individuals of *A. maculates*. Allele description: A is 100, B is 125 and C is 130.

RESULTS AND DISCUSSION

Genetic analysis of allozyme data

Six enzymes, ADH, LDH, MDH, GDH, SOD and ME, were analysed on polyacrylamide gels. No tissue specific loci were observed for the enzymes studied, the bands being identical in each case. The loci, allele frequencies and x^2 values for the differences between observed and expected Hardy-Weinberg numbers of phenotypes of each polymorphic enzyme are given in Table 1. The average heterozygosity was 2.5 and 1.46 in *O. militaris* and *A. maculatus*, respectively, while the proportion of polymorphic loci was 0.33 for both (Table 2). Of the six loci observed, only two, ADH and MDH, were found to be polymorphic in both species (Figure 1a and b). Both enzymes seem to behave as monomeric proteins. Genotypes for both species conformed to Hardy-Weinberg proportions ($p>0.05$) at these loci, with the exception of ADH locus in *O. militaris*. However, observation over a larger sample might have shown the population to be in equilibrium. Average number of alleles per locus was 1.33 and 1.5 in *O. militaris* and *A. maculatus*, respectively.

A comparison of electropherograms of isozymes reveals that the individuals are readily identifiable to species from SOD and ME loci (Figure 2). At LDH and GDH loci both species show the same common allele.

Genetic difference between the two species was estimated by calculating the Nei's genetic distance (D) using allele frequencies shown in Table 1. The D value between *O. militaris* and *A. maculatus* was quite small (D=0.6879) the genetic similarity being quite high (I=0.5026). Genetic distances are not much affected by sample size and thus a single individual from a wild

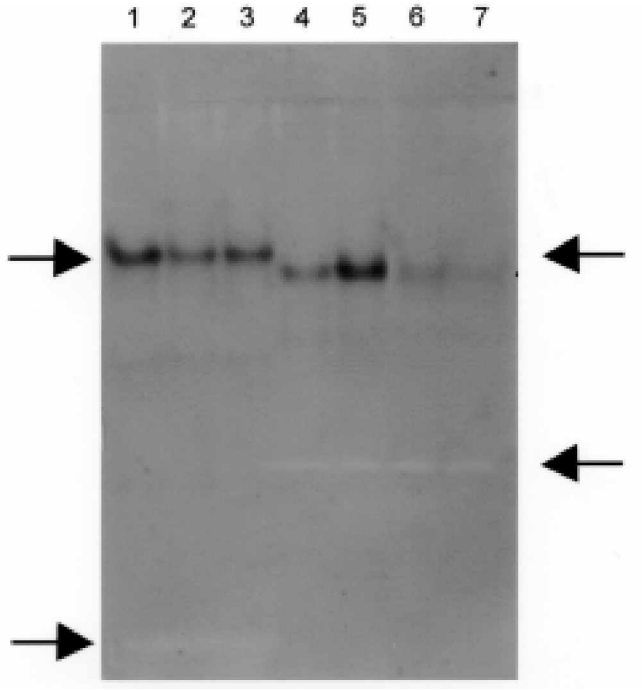

Figure 2. ME and SOD profiles in muscle tissue of *O. militaris* and *A. maculates*. Lanes 1-3: Individuals of *O. militaris*; lanes: 4-7: Individuals of *A. maculates*. Arrows indicate ME and arrowheads indicate SOD.

population may be used to represent a species (Nei, 1978). Nei (1976), estimated that for full species, D=0.10 to 2.0; sibling species which are more or less morphologically identical generally have D values two to three times smaller than the Ds between morphologically distinct species. D is generally greater than 1.0 for genera. In the family Pleuronectidae, the average genetic distance was reported as being 1.11 between genera (Ward and Galleguillos, 1983). In the family Sciaenidae, Menezes and Taniguchi (1988) and Menezes et al. (1990), reported the average genetic distance among consubfamilial genera as 1.212, ranging from 0.861 to 1.668. In Carangidae, Kijima et al. (1986), using 6 enzymes and 9 loci coding for them, indicated the average genetic distance as being 2.046 between genera, while in sparid fish Taniguchi et al. (1986), reported the average genetic distance of 0.842 between consubfamilial genera.

In this context, the genetic distance observed between *O. militaris* and *A. maculatus* in this study suggests that they would be more appropriately classified as species of the same genus rather than being assigned separate genera. The unique ability of biochemical genetic data to clarify population structure has been demonstrated both in identifying previously unknown structures as well as in falsifying previously assumed ones (Allendorf, 1987).

At the morphological level these two species are only distinguished by the presence of ossified barbels in *O. militaris*. Over twenty species of *Arius* are reported to occur in the Indian waters. The shape of the palate, the type and arrangement of teeth on the roof of the mouth cavity and the length of the barbels distinguish them. In fact, the absence of salient external characters poses difficulty in identifying them in the field. An objective way to test morphological conclusions is to use isozyme electrophoresis to obtain a measure of genetic distance, assuming that this is correlated with the closeness of common ancestry (Mckay and Miller, 1991). A biochemical investigation into the phylogenetic relationships of these species would make an interesting future study.

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