Polymorphism of Blood Plasma Esterases in Natural Population of Giant Toad, *Bufo marinus* Linn. (Amphibia: Bufonidae)

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Abstract

Starch gel electrophoretic analysis of esterases in the plasma of the giant toad, Bufo marinus *Linn., resolved three sets of electromorphs ,presumably products of three independently-segregating loci, migrating to the anodal region. These esterases were identified to be of carboxyesterase type based on the affinity to α- and β-naphthol acetates as substrates.*

Estimation of the allelic frequency revealed that all three loci showed polymorphism based on 5% criterion of polymorphism. Goodness-of-fit test between the observed genotypic frequencies with the expected frequencies in each of the EST locus showed the toad population was not at Hardy-Weinberg equilibrium. This could be attributed to heterozygote deficiency in each of the locus.

Keywords: polymorphism, plasma esterases, *Bufo marinus*

Introduction

Esterases (EC 3.1.1-) are a general class of enzymes important in the metabolism of various compounds containing ester linkages. Augustinsson (1959 a-c) demonstrated the properties of esterases in plasmata from various vertebrates using biochemical methods. It was revealed that the enzymes can be subdivided into three groups on the basis of their affinities to various substrates and selective inhibition by a number of diverse chemical agents. The three groups include arylesterase or A esterase, aliesterase or B esterase and cholinesterase or C esterase.

Esterase multiplicity has been demonstrated by Holmes and Masters (1967 a,b) which can be attributed to overlapping specificity of enzymes (Augustinsson, 1961). This group of enzymes has been assumed to be the most variable enzymes of vertebrates (Selander and Kaufman, 1973). Esterase polymorphism has been reported to be found in various vertebrate plasmata (Augustinsson, 1959 a-c; Gahne, 1966; Holmes and Masters 1968; Kuznetsov, 1995). Clinal variation in the esterase loci was demonstrated in the *Zaprionus indianus* (Parkash and Yadav, 1993), in freshwater fish, *Catostomus clarkii* (Koehn and Rasmussen, 1967; Koehn, 1969) and in the snail, *Littorina saxalis* and *L. obstusata* (Newkirk and Doyle,

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1979). Working with red blood cells of *B. marinus*, Doyungan (1995) demonstrated phenotypic differences in the esterase loci among three geographically isolated populations.

This paper describes the genetic structure of the *B. marinus* based on the plasma esterase-coding loci.

Materials and Methods

A total of 31 adult toads were obtained from the natural population in Plaridel, Misamis Occidental. Blood samples were drawn from anesthetized toads through ventricular puncture, and were fractionated in test tubes containing an anticoagulant (5% sodium citrate) by centrifugation at 4,000 revolution per minute (rpm) for 5 min. Plasmata were pipetted into clean vacutainers and were stored at -20°C.

Electrophoretic analysis of the plasma esterases was done at the Isozyme Laboratory of the International Rice Research Institute, Los Baños, Laguna, following the protocol of Glazsmann et al. (1988). Starch gel (14%) was prepared by using hydrolyzed potato starch (Sigma Chemical Company) with Tris-Citrate (Trizma base, Sigma and Citric acid monohydrate) pH 8.8 as gel buffer. Samples were loaded on the gel using filter paper wicks (Whatman filter paper no. 3). Electrophoretic run was done using Sodium Borate (pH 8.2) as electrode buffer with an initial voltage of 180 up to about 300 volts per cm for 4 hours while keeping the amperage constant at 30 mAmps. Migration of the samples was monitored based on the tracking dye.

Activity of the plasma esterases was determined in an assay solution containing Fast Blue RR in 0.5Tris-HCl (pH 7.1) and a- and β- naphthol acetates as substrates. The identification and nomenclature of the esterase-coding loci was patterned after Shaklee et al. (1990).

Results and Discussion

Analysis of the esterases activity in the zymogram 30 minutes after incubating the gel in the assay solution revealed three distinct sets of esterase electromorphs, representing three independently-segregating *EST* loci, migrating into the anodal region (Fig. 1). Based on the substrates used, these esterases belong the the carboxyesterase group. Two phenotypes (bands) were resolved for EST 1 while three each were observed for EST 2 and EST 3. Apparently, each *EST* locus is governed by codominant alleles.

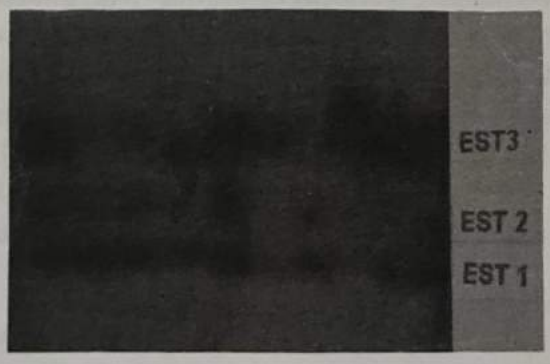

Figure 1. Zymogram of the plasma esterases of giant toad, *B. marinus*

The allelic frequency in each of the three *EST loci* is presented in Table 1. Based on the 5% criteria of polymorphism, all the *EST* loci were found to be highly polymorphic.

Table 1. Allelic frequency at the three *EST* loci in the giant toad, *B. marinus*

EST LOCUS	ALLELIC FREQUENCY		
	*1	*2	*3
EST 1	.3225	.6775	
EST 2	.3333	.5999	.0667
EST 3	.0417	.7083	.2500

Goodness-of-fit test comparing the observed zygotic frequencies with the expected zygotic frequencies showed a significant deviation from the Hardy-Weinberg equilibrium expectation of genotype frequencies. This is attributable to the great deficiency of heterozygotes among the samples (Table 2). One possible explanation to this condition is the presence of a null allele which could result in some phenotypes being classed as homozygotes when, in fact, they are heterozygotes with a null allele (Newkirk and Doyle. 1979).

Table 2. Genotype frequency and distribution at the three *EST* loci in natural population of *B. marinus*

LOCUS	GENOTYPE	NO.	FREQUENCY
EST 1	*1/*1	10	0.3225
	*2/*2	21	0.6775
		n= 31	
EST 2	*1/*1	10	0.3333
	*2/*2	16	0.5333
	*2/*3	4	0.1333
		n = 30	
EST 3	*1/*1	1	0.0417
	*2/*2	17	0.7038
	*3/*3	6	0.3500
		n = 24	

In general, enzyme polymorphism has been shown to increase the fitness among the individuals in the population by providing means of metabolic plasticity for the varying environment (Johnson, 1974). Esterases are enzymes that are highly variable so that polymorphism has been reported in the plasmata and tissue homogenates of vertebrates (Augustinsson, (1959 a-c; Gahne, 1964; Holmes and Masters, 1968; Doyungan, 1995). This group of enzymes has been demonstrated to show clinal variation/distribution (Kochn and Rasmussen, 1967; Parkash and Yadav, 1993). As such, Mateo (1971) hinted that esterases can be valuable markers in adaptation studies involving polluted waters.

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