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Electrophopretic Variation in Feral Populations of Three Freshwater Species of Fish

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Abstract

Analysis of hemoglobin and plasma esterase in the blood sample of three feral freshwater species of fish, namely, spotted gourami **(Trichogaster trichoptenus),** *mudfish* **(Channa striata)** *and catfish* **(Clarias macrocephalus)** *was conducted using horizontal starch gel electrophoresis.*

Results revealed that both the hemoglobin (HB) and plasma esterase (EST) loci in **T. tricoptenus** *showed polymorphism, consisting of two alleles as indicated by two codominantly. Migrating anodal electromorphs. For* C. striata, *only the HB locus showed polymorphism being governed by two alleles while the EST locus showed monomorphic expression. Likewise, monomorphic expression was observed in both loci in the* C. macrocephalus.

Keywords: *polymorphism, hemoglobin, plasma esterase,* T. trichoptenus, C. striata, C. macrocephalus

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Introduction

The existence of genetic variability in natural population is one important requisite for evolutionary process to occur. It self a product of evolution, generic variability plays the role of an adaptive strategy for population to cope with temporal or spatial heterogeneity of the environment. Populations living in heterogeneous habitats display large genetic variability and those living in homogenous and stable habitats are genetically depauperate (Apfelbaum et.al. 1991).

In fisheries, there has been a growing interest in assessing the extent of genetic diversity in both feral and cultured populations of fish. This is so because knowledge on the extent and nature of variability are important in the aspect of maintaining the quality of managed fish stocks (macaranas, 1991).

Gel electrophoresis is one most useful biochemical technique yet devised for studying genetic variability within and among populations of animals (Macaranas, 1991). Of the biochemical markers, hemoglobin and esterases are commonly used.

Hemoglobin is extremely an effective marker due to its heterogeneity and phylogenetically conservative nature. The electrophoretic structure of hemoglobin has been shown to be species specific (Arefjev and karnauchov, 1989). On the other hand, esterases (E.C. 3.1.1.), enzymes important in the metabolism of compounds containing ester linkages, have been widely used as a marker. This group of enzymes has been assumed to be the most variable enzymes of vertebrates (Selander and Kaufman, 1973), exhibiting clinical variation as reported in *Zaprious indiacus* (Parkash and Yadav, 1973), in toad *Bufo marinus* (doyungan and Barrion, 1997; Doyungan, 1997) and in the freshwater fish *Catostomus clarkii* (kochn and Rasmussen, 1967; Kochn, 1969).

This paper describes the occurrence of genetic variability in the presumptive loci of hemoglobin and plasma esterase in feral populations of freshwater fishes, namely: spotted gourami (*T.trichoptens*), mudfish (*C. striata*) and catfish (*C. macrocephalus*).

Materials and Methods

Thirty specimens each of the three freshwater fish species were obtained from the natural populations in Tibanga, Iligan city (for gourami and mudfish in November 1997) and in Lala, Lanao del Norte (catfish in November 1998). Blood sample were collected from decapitated live fish usiong Pasteur's pipet and were placed in eppendorf tubes containing an anticoagulant (5% sodium citrate). The samples were fractionated by centrifugation at 4,000 revolution per minute (rpm) for 5 min at 4°C. Plasmata were pipetted into new eppendorf tubes and were stored at 20 C. The erythrocytes were washed twice with physiological saline solution prior to storage at 2°C.

Electrophoretic analysis of the blood proteins was done using a MUPID 2 Mini Gel Electophoresis at the Department of Biological Sciences, MSU-IIT. For hemoglobin, 12 percent hydrolyzed potato starch (Sigma chemical Company) was used in the preparation of gel with Tris-Borate-EDTA (ph 8.9) as gel buffer. The same buffer was also used as electrode buffer (continuous buffer system). Foe plasma esterase, 1.3% starch was prepared using Tris-citrate (pH 8.6) gel buffer and Borate (pH 8.0) as electrode buffer. Frozen samples of

erythrocytes and plasmata were thawed thoroughly before loading. About 3 parts of distilled water were added to the erythrocyte pellet to lyze the cells. About 10 of the samples were loaded on the gel Electrophoretic run was done at 50 volts/cm for 3 hours.

Hemoglobin was deterimed by staining the gel with 1% buffalo Black (Amado Black) followed by several washing of the gel with destaining solution (method-distilled water - acetic acid, 5:5:1 v/v). Activity of the plasma esterase was determined by incubating the gel on an assay solution containing Fast Blue RR in 0.5M Tris HCl (pH 7.1) and a and B naphthol acecates as substrates. The identification and nomenclature of the protein-coding loci was patterned after Shaklee et.al. (1990).

Results and Discussion

The diagrammatic electrophoretogram patterns of hemoglobin (HB) in the three feral species of freshwater fish are shown in Figure 1. Two codominantly-segregating anodal bands were resolved for both *T. trichpterus* and *C. striata* while a single anodal band was found for *C. macrocephalus*. As such, the presumptive *HB* locus in the gourami is governed by two alleles, represented as **1* and **2* with the respective allelic frequency of 0.148 and 0.852. Likewise, two alleles govern the HB locus in the mudfish, similarly represented as **1* and **2*, with the allelic frequency of 0.74 and 0.26, respectively. This locus, however, is fixated to a single allele (*1) in the catfish population. The data on the genetic structure of the three populations are summarize in Table 1.

Table 1. *Data on the genetic structure based on the HB locus of the feral populations of three freshwater species of fish.*

SPECIES	NO. OF ALLELES	NO. OF PHENOTYPES GENOTYPES	POLYMORPHISM*
T. trichoptenus	2	2	Polymorphic
C. striata	2	3	Polymorphic
C. macrocephalus	1	1	Monomorphic

*Based on 5% criterion of polymorphism

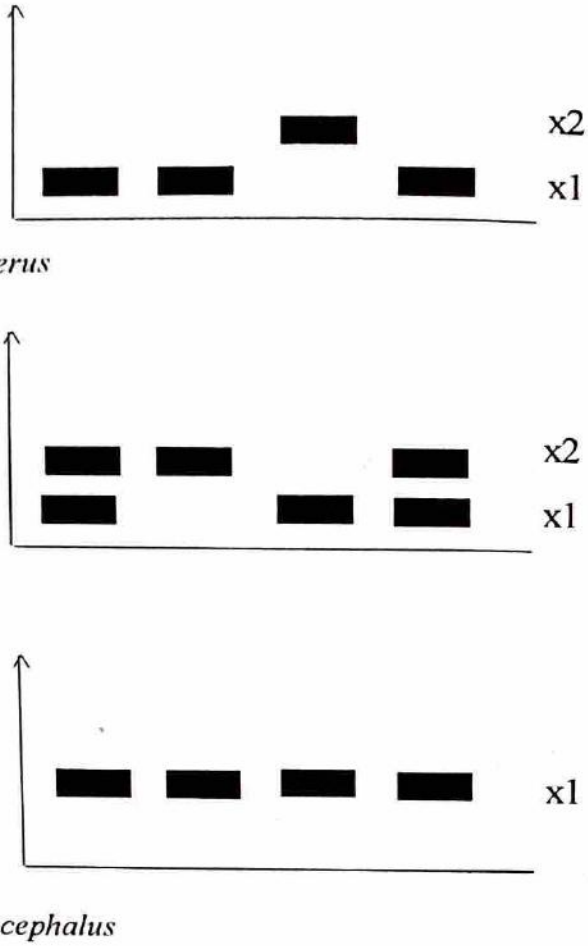

Figure 1. *Diagrammatic representation of the hemoglobin (HB) phenotypes in the freal populations of three species of freshwater fish.*

The *HB* locus in both the gourami and mudfish exhibited polymorphic expression based on 5% criterion.

Arefjev and karmauchov (1989) reported that HB in three Abramis species is a taxonomically informative protein, and its structure is highly species-specific. This species specifically characteristics of HB expression, however can not be determined in the three species of freshwater fish used in this study in the absence of available data.

Analysis of the estates activity in the zymogram following incubation of the gel on the assay solution revealed two distinct anodal esterase electromorphus in the gourami while only a single band resolved for both the mudfish and catfish (Fig. 2). Apparently, the plasma esterase protein is a product of a single EST locus. The EST in the gurami is governed by two alleles, represented as *1 and *2, with the respective frequency of 0.75 and 0.25. In both the mudfish and catfish, this locus was fixated to a single allele (*1). Data on the genetic structure of the population based on the EST locus is presented in Table 2.

Table 2. Data on the genetic structure based on the EST locus of the feral population of three freshwater species of fish.

SPECIES	NO.OF ALLELES	NO. OF PHENOTYPES GENOTYPES	POLYMORPHISM*
T. trichopterus	2	3	Polymorphic
C. striata	1	1	Monomorphic
C. macrocephalus	1	1	Monomorphic

*Based on 5% criterion of polymorphism

Obviously, the EST in the spotted gourami only showed polymorphism.

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). Some factors, however, may influence the activity and hence the adaptability of certain alleles in a locus. The electrophoretic variability or lack of it in both the HB and EST loci in the three species freshwater fish in this study may be influenced largely by both the individual variability at one locus from genetic segregation (Kamiski, et al., 1987) and interlocus variation that may have been induced by gene substitution, mutation and natural selection in the locus (Nei, 1975).

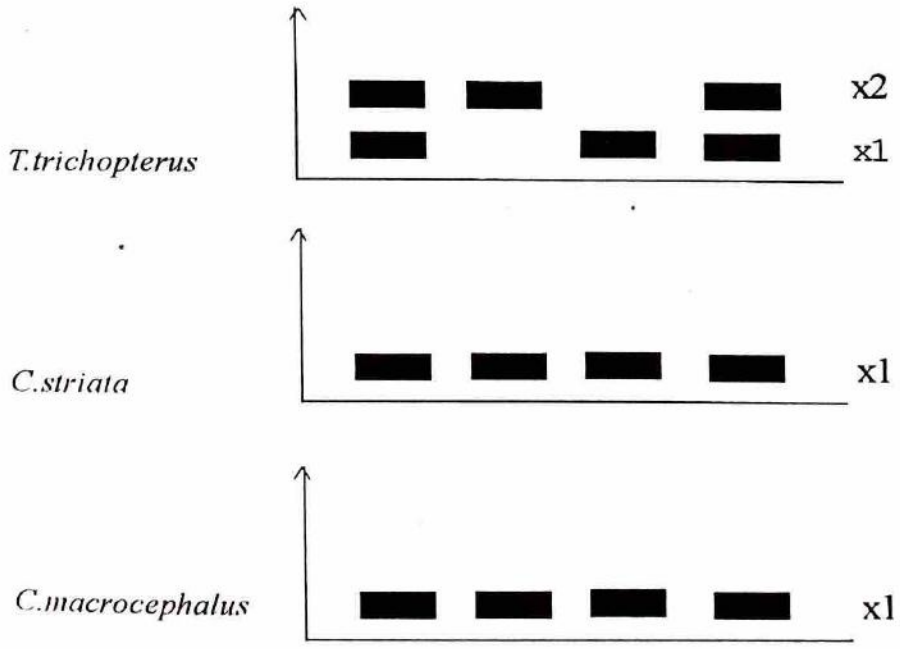

Figure 2. Diagrammatic representation of the plasma esterase (EST) phenotypes in the freal populations of three species of freshwater fish.

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