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Esterase Heterogeneity in Selected Aquarium Fishes

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Abstract

This study was conducted to determine biochemical genetic diversity between commonly reared aquarium fish species. Esterase gene loci were evaluated based on protein products elucidated by electrophoresis. There were 11 different Isozymes identified based on the relative mobility of the bands. Based on esterase patterns, the different species were observed to be genetically-differentiated. Fish species were observed to differ from each other based on Isozymes expressed.

Introduction

Variation is the fundamental property of living organisms. The existence of variation among biological system is a necessity for evolution. To understand how a population evolves, we must begin with the variation in traits that exists among individuals.

Variation can be directly observed by the difference in morphological structures, color and behavior of different species. But species of the same kind displays similar superficial characteristics and properties in which their variation exist in their biochemical aspects.

Biochemical studies are being conducted in order to verify that there are possible genetic, variations in the expression of the biochemical components of species.

Different species of aquarium fishes displays readily discernable variation of superficial characteristics. The most commonly observable characteristics are the color, shape of the fin, and gross body length. But even with the obvious differences between its morphological characteristics, these aquarium fishes feed in the same fish foods when placed in novelty aquariums.

A biochemical genetic study was conducted whether variability in morphology are likewise displayed in its genetic background. Esterase genes are specifically investigated by the pattern on products shown in gels through electrophoresis. this approach had been used in investigating genetic relationships of not only morphologically distinct species but within a population of species as well.

The ten aquarium species subjected to esterase assay were the commonly known

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species Black Tetra (*Gymnocorymbus ternetzi*), Tiger Barb (*Barbus phutonio*), Guppy (*Poecilia reticulata*), Silver Molly (*Poecilla mexicana*), Platy (*Xiphophorus maculatus*), Red Jewel (*Hyphessobryron callistus*), Swordtail (*Xiphophorus helleri*), Rosy Barb (*Barbus vittatus*), Convict (*Acathurus triostegus*), Albino Red Cross (*Hemigrammus stictus).*

Agarose Gel Electrophoresis was used to determine genetic variability among the ten chosen aquarium fishes, using esterase as a maker. Genetic variability was observed by assessing band patterns through the different migratory patterns of the enzyme.

Materials and Methods

The samples used were purchased from a hobby shop that sells aquarium fishes. Ten different species were bought and brought to the College of Sciences and Mathematics' Entomology laboratory. The ten different species were identified, weighed and its gross body length were measured and recorded.

Preparation of Samples

The samples was then homogenized in a glass homogenizer containing 1.0 mL of 1% Glycerol at low temperature. The temperature was maintained by soaking the glass homogenizer in a plastic container filled with ice. The glass homogenizer was then washed properly after each species by using distilled water and detergent.

Homogenized samples were then placed in microcentrifuge tubes soaked in ice. The samples where then centrifuged at 4 degrees Celsius for 15 minutes by using centrifuge machine. The resulting supernatant was decanted to remove cellular debris. The decanted supernatant was then centrifuged again. The supernatant was again decanted.

Electrophoretic Analysis

Esterase patterns of the 10 species of aquarium fishes were studied by agarose gel electrophoresis. Brothymol blue (tracking dye) and 10 microliter of supernatant from each species was loaded into the agarose gel and immersed in a mini-gel electrophoresis setup containing Electrode buffer. The electrophoresis setup was subjected to a 50 V volatage. The mini-gel electropheresis setup was soaked in ice. The mini-gel electrophoresis setup was turned off after 2.5 hours. The agarose gel was stained using a Fast Blue RR as coupler dye for 2 hours

The number and the distance traveled by the bands from the wells were recorded with reference to the distance traveled by the tracking dye. Esterase Isozymes was studied using a schematic diagram drawn from the accrual gel. Isozymes were labeled and similar bands were recorded. Similarity indices were computed by using formula:

$$\text{Similarly index} = \frac{\text{no. of similar bands}}{\text{No. of dissimilar bands} + \text{no. of similar bands}}$$

Results and Discussions

The ten species of aquarium fishes were identified as Black Tetra (*Gymnocorymbus ternetzi*), Tiger Barb (*Barbus phutonio*), Guppy (*Poecilia reticulata*), Silver Molly (*Poecilla mexicana*), Platy (*Xiphophorus maculatus*), Red Jewel (*Hyphessobryron callistus*), Sword-tail (*Xiphophorus helleri*), Rosy Barb (*Barbus vittatus*), Convict (*Acathurus triostegus*), Albino Red Cross (*Hemigrammus stictus*). The scientific names, weight, length, and color are summarized in Table 1. The description of the fishes studied only focuses on the color to basically express the existence of variability of the fishes in the superficial aspect. The data gathered regarding on the color alone, implies the variability of the species being studied. The color of the species, alone, basically introduces us to the fact that, indeed, there is the existence of genetic differences among these species. But the assumption could be considered vague, since the genetic differences cannot be fully justified by the colors alone. It could rather be useful to observe and discern according to their taxonomical relationship and similarity on biochemical components.

Table 1 shows that the pairs Tiger Barb and Rosy Barb, Australian Guppy and Silver Molly, Platy and Swordtail belongs to the same Genus *Barbus*, *Poecilla*, and *Xiphophorus* respectively. The species did not seem to exhibit any close resemblance from their colors even from the fact that there are species that belongs to the same genus. The implication of this data shows that variability already exist even among the genus level with respect to their body coloration.

Table 1. *The Recorded Common Name, Scientific Name, Weight, Gross Body Length and Color of the Ten Species of Aquarium Fishes Studied.*

SPECIMEN NO.	COMMON NAME	SCIENTIFIC NAME	WEIGHT (g)	GROSS BODY LENGTH (cm)	COLOR
1	Black Tetra	*Gymnocorymbus ternetu*	1.8	5.0	Grayish, Light Brown
2	Tiger Barb	*Barbus phutunio*	0.7	3.3	Gray with Black Vertical lines
3	Australian Guppy	*Poecilia reticulata*	0.4	3.0	Yellowish at its dorsal part and Grayish at its ventral part
4	Silver Molly	*Poecilia mexicana*	0.7	3.5	Silver
5	Platy	*Xiphophorus maculatus*	0.4	2.8	Grayish with a distinct black spot at its dorsal tail
6	Red Jewel	*Hyphessobrycon callistus*	0.7	3.3	Combination of Gray and black with three distinct isolated black spots
7	Swordtail	*Xiphophorus helleri*	0.5	3.5	Orange
8	Rosy Barb	*Barbus vittatus*	0.9	4.0	Metallic Silver
9	Convict	*Acathurus triostegus*	0.5	3.1	Dark Yellow
10	Albino Red Cross	*Hemigrammus stictus*	0.4	3.5	Fins are orange; Pearl orange body

The initial indication of variability of the ten species is further supported by electrophoretic data of esterase Isozymes resolved from agarose gel electrophoresis.

The electrophoretograms (Fig. 1) of the gel show eleven isozymes of esterase. The existence of variation among the species was showed in the qualitative observation of the Relative Migration Pattern (rmp) of the isozymes.

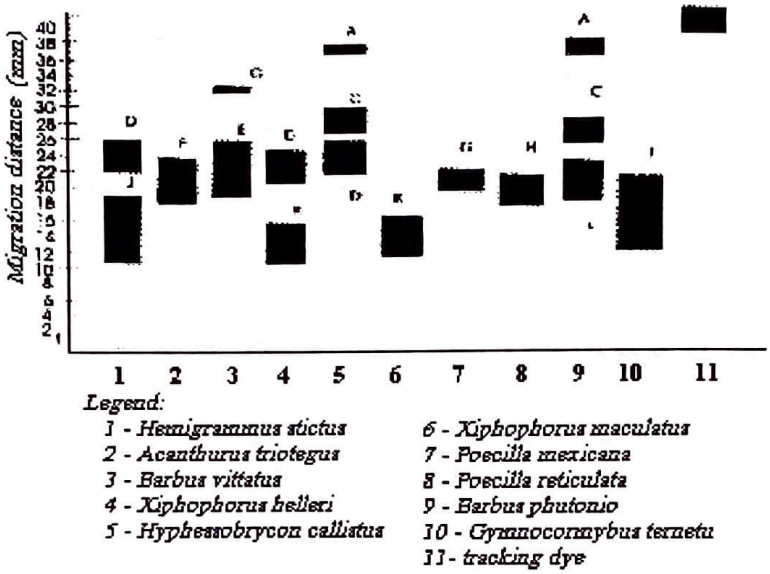

Figure 1. *Electrophoretograms of esterase Isozymes in ten species of aquarium fishes.*

In Figure 1, isozymes I, H, G, E, and J are specific to *Gymnocorymbus ternetu, Poecilla reticulata, Poecilla mexicana, Barbus vittatus,* and *Hemigrammus stictus* respectively. It can be seen in Figure 11 that even the species of the same Genus varies with their isozymes content and migration pattern. This variation of isozymes between the same Genus is exhibited by *Barbus phutunio* and *Barbus vittatus.*

It was further observed that *Barbus phutunio* and *Hybhessbrycon callistus* have highly similar bands even from the fact that these two species belongs to two different genus. Isozyme A *Barbus phutunio* is observed to be more anodic than with that of A of *Hyphessbrycon callistus* but Isozyme C of *Hyphessbrycon callistus* seem to be more anodic than with Isozyme C of *Barbus phutunio.* It is also observed that Isozyme D of *Hyphessbrycon callistus* seems to be similar to Isozyme F of *Barbus phutunio,* but Isozyme D is more anodic.

Species that belong to genus *Poecilia* seem to display similar bands than with the species that belongs to genus *Barbus.* It can be readily discernable by looking at the electrophoretogram that Isozyme G of *Poecilla mexicana* is more anodic that isozyme H of *Poecilla reticulata,* although it can be assumed that these isozymes belong to only one gene loci. Isozyme D of *Hyphessobrycon callistus* and *Hemigrammus stictus* displays the same mobility and they are more anodic than Isozyme D of *Xiphophorus helleri.* Isozyme K of *Xiphophorus maculatus* displays faster mobility compared to Isozyme K of *Xiphophorus helleri.*

Table 2. *Relative Mobility of Esterase Among the Ten Species of Aquarium* ***Fishes***

Species	Isozymes	Band Range		Distance From the	Travelled Well (mm)	R_f
		Lower	Upper	Isozymes	Tracking Dye	
Gymnocormybus ternetu	I	1.5	2.2	18.5	40.0	0.46
Barbus phutunio	F	1.9	2.4	21.5	40.0	0.54
	C	2.6	2.9	27.5	40.0	0.69
	A	3.7	3.9	38.0	40.0	0.95
Poecilla reticulata	H	1.8	2.2	20.0	40.0	0.50
Xiphophorus mexicana	G	2.0	2.3	21.5	40.0	0.50
Xiphophorus maculatus	K	1.2	1.7	60.0	40.0	0.36
Hyphessobrycon callistus	D	2.2	2.6	24.0	40.0	0.60
	C	2.7	3.0	28.5	40.0	0.70
	A	3.7	3.8	19.0	40.0	0.47
Xiphophorus helleri	K	1.1	1.6	13.5	40.0	0.34
Barbus vittatus	D	2.1	2.5	23.0	40.0	0.57
	E	1.9	2.6	22.5	40.0	0.56
Acathurus triotegus	G	3.2	3.3	32.5	40.0	0.80
	F	1.8	2.4	21.0	40.0	0.52
Hemigrammus stictus	J	1.1	1.9	15.0	40.0	0.37
	D	2.2	2.6	24.0	40.0	0.60

The existence of various isozymes resolved by electrophoresis can be attributed to gene duplication. By virtue of biological evolution, it is a fact that all complexities that developed have their origins from much simpler forms. Gene duplication is a process and evidence for the existence of evolution. The presence of several gene loci coding for a particular enzyme indicates the presence of a more highly complex physiological activity. These developments often occur when there is a need for further development of biochemical factors for the survival of a certain species.The relative mobilities (Rf) of the different isozymes supports the genetic basis of variation among the ten species (Table 2). The presence of these gene loci duplication among the species actually tells us that these species tend to adapt to whatever stress that it may encounter during their evolution process.

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