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ELECTROPHORETIC VARIATIONS IN ISOESTERASES OF THE TWO CATFISH SPECIES, CLARIAS GARIEPINUS AND CLARIAS ANGUILLARIS, IN THE EGYPTIAN FRESHWATER

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Key words: Esterase, Genetic distance, Catfish, Egypt.

ABSTRACT

Esterase isozymes were compared electrophoretically, using three organs; liver, spleen and kidney to determine the phylogenetic relationship between Clarias gariepinus and C. anguillaris, as well as, to know the genetic differences between two types of C. gariepinus to verify their genetic isolation by estimating the genetic distance between types and /or species of genus Clarias. The results indicated that the genes controlled esterase isozymes were six loci in C. gariepinus and five loci in C. anguillaris, whereas the locus Est-2C was completely absent in all organs of C. anguillaris. Moreover, anodal loci were the only ones that expressed in all studied samples, and the locus Est-2 A showed higher activity than other loci in all specimens.

The overall genetic identity and genetic distance between these tow species were 0.81 and 0.19 respectively. The maximum magnitudes of genetic identity (GI=0.99) and minimum genetic distance (GD=0.01) were found between types of C. gariepinus. The lowest genetic identity between C. anguillaris and type A (GI= 0.87) and type B (GI= 0.80) were appeared in spleen, while the lowest genetic distance (GD=0.02) between them were found in kidney and liver, respectively. The combined genetic identity and genetic distance between types A and type B of C. gariepinus and C. anguillaris were not similar, whereas they equal GI = 0.81, GD = 0.19 and GI = 0.78, GD = 0.22 respectively.

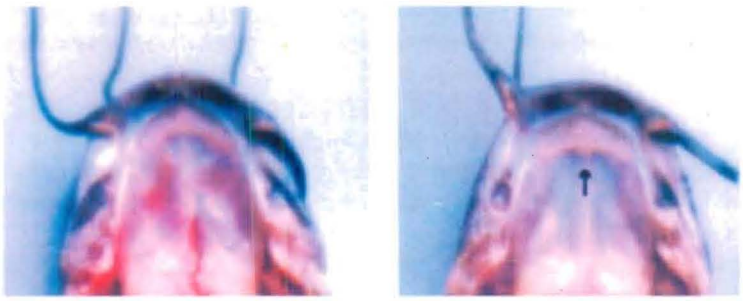
INTRODUCTION

Catfishes are one of the most important food fish in Egypt, since they constitute about 12.43% of total annual freshwater catch of the country (GAFRD, 2000).

The taxonomy of African catfish genus *Clarias* has been extremely confusing for a long time. Teugles (1982) has given a new systematic revision of the African catfish of

this genus; he reported that *Clarias lazera* is a strain of *C. gariepinus* .So the name *C. gariepinus* is at present generally accepted.

According to Boulenger (1915) in his revision of the Nile fishes confirmed that there are two catfish species, *C. lazera* and *C. anguillaris* in Egyptian freshwater. Moreover he had pointed out a marked difference in vomerine teeth shape within *C. lazera* (C. *gariepinus)* species. Whereas, these vomerine teeth in this species were granular, forming a crescentic band with or without a posterior median process and their greatest width in the middle reaching from one and half to two and half of the premaxillary band. Bakhoum (1996) studied inter-specific variation within the African catfish (*C. gariepinus*), his results indicated that this species is divided into two distinct morphotypes varied in the shape of vomarine teeth: without posterior median process (type A) or with posterior median process (Type B) (Fig.1). This conclusion is supported by significant differences in morphometric measurements and variations in egg size between the two fish types.



Type (A) **Type (B)**

Fig.1. Morphotypes of *Clarias gariepinus*: Type A without a posterior median process and type B with a posterior median process.

Identification of *Clarias* species according to their morphological differences is not completely satisfactory, while electrophoretic methods have proved to be useful in species identification (Allendorf and Utter, 1979). Furthermore, according to Tave and Smitherman (1980) and Menezes *et al.* (1993), the electrophoresis can give an independent estimate of the level of variation within a population without an extensive survey of morphological and other quantitative traits.

The present study analyze electrophoretically esterase isozyme in order to reveal biochemical genetic markers and to find out the phylogenetic relationship between *C. gariepinus* and *C. anguillaris*, as well as, to know the genetic differences between two types of *C. gariepinus* to verify their genetic isolation by estimating the genetic distance between types and /or species of genus *Clarias*.

MATERIAL AND METHODS

Specimens of *Clarias gariepinus* and *C. anguillaris* were collected from the commercial catch at El-Maadia fish center in Lake Edku from August 2001 to November 2002.

Agar- Starch-Polyvinyl Pyrolidine (P.V.P.) gel electrophoresis was carried out according to the procedures described by Shaw and Kaen (1967); El-Metainy *et al.* (1977) and Sabrah and El-Metainy (1985). For electrophoretic esterase analysis, liver, spleen and kidney were taken from 115 (59 type A and 56 type B) and 41 live specimens of *C. gariepinus* and *C. anguillaris* respectively. The fish size of the two species ranged between 180-450 mm in total length.

According to the results of esterase electrophoretic patterns, gene frequency of alleles segregating at each locus and in combined organs tissues were estimated. The identity of genes and genetic distance were estimated between each species and type according to Nei (1971).

RESULTS

The electrophoretic pattern of esterase isozymes from liver, spleen and kidney of different types of *Clarias gariepinus* indicated that four anodal and two cathodal loci controlled esterase isozymes. While four anodal and only one cathodal loci controlled this isozymes in *C. anguillaris*. Moreover, anodal loci were the only ones that expressed in all studied samples, and the locus Est-2 A showed higher activities than other loci in all examined specimens.

Esterase isozyme genes were different in homozygosity or heterozygosity in the two species. The loci Est-1 A, Est-3 A, Est-1C and Est-2C showed homozygous alleles, while Est-2 A and Est-4 A had heterozygous alleles in all organs. The genes controlled these isozymes were six loci in *C. gariepinus* and five loci in *C. anguillaris*, whereas the locus Est-2C were completely absent in all organs of *C. anguillaris* (Fig.2).

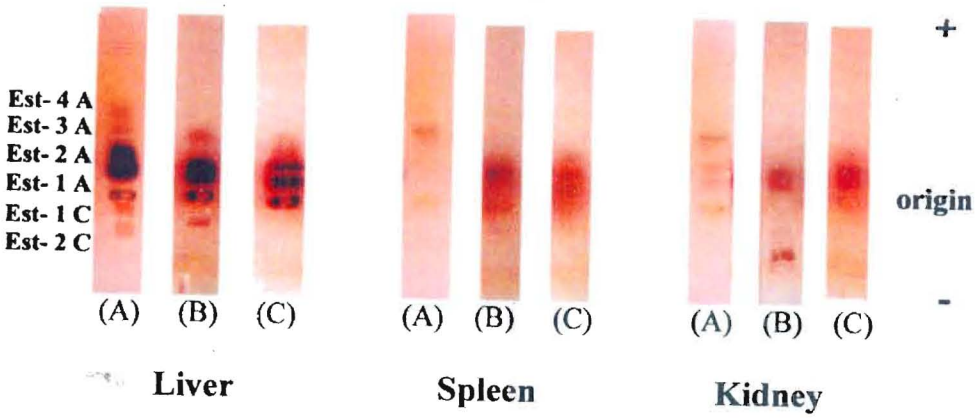


Fig.2. Esterase zymogram extracts from liver (L), spleen (S) and kidney (K) of different morphotypes of *Clarias gariepinus* and *C. anguillaris*.

Gene frequency of liver esterase isozyme for alleles segregating at different loci revealed that Est-2C was expressed in type A of *C. gariepinus*, while Est-1C was the invariable expression in relation to type and / or species (Table 1). In spleen the specificity of loci expression, it was noted that Est-2 A and Est-1C were the only loci invariable expression, while Est-4 A, Est-3A and Est-1A showed variable expression. Moreover the Est-2C was completely absent in spleen of both examined species. Est-1C was specific for Types (A and B) of *C. gariepinus*, which was absent in the spleen of *C. anguillaris* (Table 2).

Data of esterase isozymes from kidney indicated that the Est-2C locus was limited to Type B of *C. gariepinus* only. Est-3A revealed variable expression, while other loci showed invariable expression in kidney tissue for examined species (Table 3).

With regard to the gene frequency estimations for esterase alleles in combined mentioned tissues, revealed that Est-2C locus was limited to *C. gariepinus*, which found in types A and B of this species (Table 4).

Genetic identity (GI) and Nei's genetic distance (GD) between *C. gariepinus* and *C. anguillaris* derived from separate and combined organs are shown in Table (5). This table declares that genetic identity ranged from 0.86 in spleen to reach the maximum value 0.90 in liver. The minimum value of genetic distance (0.10) was derived from liver tissues, while the maximum one (0.14) was in spleen. The overall genetic identity and genetic distance between these tow species were 0.81 and 0.19 respectively.

Table 1 : Gene frequency estimates for alleles segregating at different loci coding for liver esterase isozymes of *Clarias gariepinus* and *C. anguillaris* from Lake Edku.

Species	Loci											
	Est-4 A		Est-3 A		Est-2 A		Est-1 A		Est-1C		Est2C	
	S	F	+	-	S	F	+	-	S	F	+	-
Clarias gariepinus	0.490	0.510	0.807	0.193	0.532	0.468	0.477	0.523	0.073	0.927	0.009	0.991
C. anguillaris	0.600	0.400	0.710	0.290	0.517	0.483	0.480	0.517	0.097	0.903	---	---
C. gariepinus (Type A)	0.575	0.425	0.833	0.167	0.545	0.455	0.444	0.556	0.070	0.926	0.019	0.981
C. gariepinus (Type B)	0.429	0.571	0.782	0.218	0.481	0.519	0.509	0.491	0.073	0.927	---	---

Table 2 : Gene frequency estimates for alleles segregating at different loci coding for liver esterase isozymes of *Clarias gariepinus* and *C. anguillaris* from Lake Edku.

Species	Loci											
	Est-4 A		Est-3 A		Est-2 A		Est-1 A		Est-1C		Est2C	
	S	F	+	-	S	F	+	-	S	F	+	-
Clarias gariepinus	0.667	0.333	0.624	0.376	0.390	0.610	0.266	0.734	0.046	0.954	---	---
C. anguillaris	0.833	0.167	0.452	0.548	0.375	0.625	0.226	0.774	---	---	---	---
***C. gariepinus* (Type A)**	0.750	0.250	0.704	0.296	0.375	0.625	0.185	0.815	0.019	0.981	---	---
***C. gariepinus* (Type B)**	0.500	0.500	0.546	0.454	0.403	0.597	0.346	0.654	0.073	0.927	---	---

Table 3 : Gene frequency estimates for alleles segregating at different loci coding for liver esterase isozymes of *Clarias gariepinus* and *C. anguillaris* from Lake Edku.

Species	Loci											
	Est-4 A		Est-3 A		Est-2 A		Est-1 A		Est-1C		Est2C	
	S	F	+	-	S	F	+	-	S	F	+	-
Clarias gariepinus	0.646	0.354	0.426	0.574	0.500	0.500	0.505	0.495	0.064	0.936	0.009	0.991
C. anguillaris	0.667	0.33	0.710	0.290	0.441	0.559	0.516	0.484	0.032	0.968	---	---
C. gariepinus (Type A)	0.636	0.364	0.426	0.574	0.510	0.490	0.519	0.481	0.07	0.926	---	---
C. gariepinus (Type B)	0.654	0.346	0.546	0.454	0.491	0.509	0.491	0.509	0.055	0.945	0.018	0.982

Table 4: Gene frequency estimates for alleles segregating at different loci coding for esterase isozymes in combined organs (Liver, spleen and kidney) of *Clarias gariepinus* and *C. anguillaris* from Lake Edku.

Species	Loci											
	Est-4 A		Est-3 A		Est-2 A		Est-1 A		Est-1C		Est2C	
	S	F	+	-	S	F	+	-	S	F	+	-
Clarias gariepinus	0.560	0.440	0.639	0.361	0.474	0.526	0.416	0.584	0.061	0.939	0.006	0.994
C. anguillaris	0.658	0.342	0.624	0.376	0.440	0.560	0.409	0.591	---	---	---	---
C. gariepinus (Type A)	0.628	0.372	0.654	0.346	0.493	0.507	0.383	0.617	0.056	0.944	0.006	0.994
C. gariepinus (Type B)	0.500	0.500	0.624	0.376	0.480	0.520	0.449	0.551	0.067	0.933	0.006	0.994

Table 5 : Estimates of genetic identity and genetic distance between *Clarias gariepinus* and *C. Anguillaris* based on different loci coding for esterase isozymes in liver, spleen, kidney and combined organs.

Species	Genetic identity				Genetic identity			
	Clarias gariepinus				*Clarias anguillaris*			
	Liver	Spleen	Kidney	Combined	Liver	Spleen	Kidney	Combined
Clarias gariepinus	---	---	---	---	0.101	0.141	0.113	0.191
Clarias anguillaris	0.899	0.858	0.887	0.810	---	---	---	---

The genetic identity and genetic distance between *C. anguillaris* and *C. gariepinus* (types A and B) varied in wide range, the lowest values of genetic identity between *C. anguillaris* and type A (GI= 0.87) and type B (GI= 0.80) were appeared in spleen, while the lowest genetic distance (GD=0.02) between *C. anguillaris* and type A and type B of *C. gariepinus* were found in kidney and liver, respectively.

The maximum combined genetic identity GI= (0.99) and minimum genetic distance (GD= 0.01) were found between the two types of *C. gariepinus* (A and B). The combined genetic identity and genetic distance between the type A and type B of *C. gariepinus* and *C. anguillaris* were not similar, whereas they equal GI = 0.81, GD = 0.19 and GI = 0.78, GD = 0.22 respectively (Table 6).

DISCUSSION

The use of molecular genetics is increasingly contributing to our knowledge of fundamental issues in evolutionary biology of aquatic organisms (Bernatchez and Duchesne, 2000). For determining the role of evolution forces involved in population divergence and, ultimately, speciation events (Bernatchez *et al.* 1999 and Lu & Bernatchez, 1999).

However, the esterase isozymes as the active proteins has been extensively used as a genetic marker to separate populations and postulated sibling species and to study genetic variability among populations (Klemetsen *et al.*, 1985 and Hindar *et al.*, 1986).

In the present study the gene frequency and genetic distance are estimated for alleles segregation at different loci coding for esterase isozymes in liver, spleen and kidney, which revealed the highest activity units more than other organs (Ali, 1994). The specificity of isozymes to types and species were indicated by the Est-2C loci, which its presence was restricted to liver of type A and kidney of type B of *Clarias gariepinus* and completely absent in all examined organs of *C. anguillaris*. The number of esterase isozyme bands had different epigenesis at the level of isozymes and allozymes between the two species. This finding agrees with Ali (1994) who mentioned that the genes controlled these isozymes were more in *C. lazera* than in *C. anguillaris*.

The genetic distance between the two species ranged from 0.10 to 0.19, which revealed the great genetic divergence between them. The maximum magnitudes of genetic identity (GI=0.99) were found between the two types of *C. gariepinus*. The genetic distance between the two types of *C. gariepinus* and *C. anguillaris* were not varied (0.19-0.22), and the less genetic distance estimated between *C. anguillaris* and types A and B (0.02) derived from kidney and liver respectively.

Table 6 : Estimates of genetic identity and genetic distance between types A and B of *Clarias gariepinus* and *C. anguillaris* based on different loci coding for esterase isozymes in liver, spleen, kidney and combined organs

Species	*Clarias anguillaris*				*C. gariepinus* (Type A)				*C. gariepinus* (Type B)			
	Liver	**Spleen**	**Kidney**	**Comb.**	**Liver**	**Spleen**	**Kidney**	**Comb.**	**Liver**	**Spleen**	**Kidney**	**Comb.**
Clarias anguillaris	---	---	---	---	0.096	0.125	0.023	0.187	0.017	0.201	0.099	0.221
C. gariepinus (Type A)	(0.904)	(0.874)	(0.977)	(0.813)	---	---	---	---	0.0101	0.055	0.096	0.012
C. gariepinus (Type B)	(0.983)	0.799)	(0.900)	(0.780)	(0.900)	(0.946)	(0.904)	(0.988)	---	---	---	---

The genetic identity is given in parentheses.

These results are considered as a spotlight on the evolution concept; it revealed the important implication of the esterase polymorphism in discrimination variations between types and / or species.

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