

Genetic Evidence for Natural Hybridization Between Nile Tilapia (*Oreochromis niloticus*; Linnaeus, 1757) and Blue Tilapia (*Oreochromis aureus*; Steindachner, 1864) in Lake Edku, Egypt

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Abstract: This study was conducted to determine the hybrid nature of tilapias intermediate morphological forms by using esterase isozymes electrophoretic analysis and hybrid index. Esterase isozymes in liver and kidney were electrophoretically compared, to determine the phylogenetic relationship between the intermediate morphological forms and their putative parents, *Oreochromis niloticus* and *Oreochromis aureus*. Also, the hybrid index was estimated to verify their hybrid nature. Results indicated that the genes controlled esterase isozymes are five loci in both organs of *O. niloticus* and hybrid types, while they were four in liver and five in kidney of *O. aureus*, whereas, the locus Est-4A was completely absent from the liver of this species. Moreover, Est-1C was the only cathodal locus, which present in all organs of examined fishes. The overall genetic identity and genetic distance between these two species were 0.862 and 0.148, respectively. Comparing the hybrid forms with parental species revealed that, the magnitudes of maximum genetic identity (GI=0.962) and minimum genetic distance (GD=0.039) were found between hybrid 3 and *O. niloticus* followed by relation between hybrid 1 and *O. aureus* (GI=0.937, GD=0.066). While, these values between hybrid 2 and both *O. niloticus* (GI=0.956, GD=0.045) and *O. aureus* (GI=0.897, GD=0.109) were found to be intermediating the relationships between parental species and hybrid types. Using maximum-likelihood to estimate the hybrid index revealed that these hybrid forms (H1, H2 and H3) have 81, 80 and 79% affinity with the parental species, respectively.

Key words: Genetic distance • Natural hybrids • Tilapia • Lake Edku • Egypt

INTRODUCTION

Tilapias have a great economic importance in the Egyptian fisheries; they constitute about 39.9 % of the total catch of Egyptian water and 82.4 % of the total catch of Lake Edku [1]. In all Egyptian brackish Lakes, specimens of tilapia were found with external appearance intermediate to Nile tilapia (*Oreochromis niloticus*) and blue tilapia (*Oreochromis aureus*). The morphological features of these specimens are similar to the features of artificial hybrids, which obtained from crossing between *O. niloticus* and *O. aureus* [2]. Moreover, it was confirmed by examination of the biochemical characteristics of soluble muscle proteins and amino acids content that these suspected natural hybrid specimens in Lake Edku resulted from crossing between male of *O. niloticus* and female of *O. aureus* [3]. In addition, [4] got evidence by methods of biometrical analysis for the existence of natural hybridization between Nile tilapia, *O. niloticus* and blue tilapia, *O. aureus* in Lake Edku.

Several investigators have documented the apparent case of natural hybridization between different species of tilapias [5 -7]. The natural hybrid reveals the high heterogeneity of its different traits, which are inherited from different parents and poses the question of how to recognize the different hybrid forms, such as the F1 generation and backcrossing specimens from the parental species.

Electrophoresis methods have proved to be useful in species identification [8]. It can give an independent estimate of the level of variation within a population without an extensive survey of morphological and other quantitative traits [9, 10]. Esterases were investigated in several organisms as useful genetic markers in tissue differentiation, population variations and species identification, whereas, [11] employed esterases as genetic markers in red tilapia and [12, 13] successfully used esterase isozymes for identification of tilapia species.

In the present study, esterase isozymes analysis was carried out in order to reveal biochemical genetic markers and to find out the phylogenetic relationships between the putative parents, *O. niloticus* and *O. aureus* and the suspected hybrid forms by estimating the genetic identity and hybrid index. In addition, to test the hypothesis that hybridization explains the intermediate morphological forms, which found in Lake Edku.

MATERIAL AND METHODS

Specimens of *O. niloticus* (N=64) *O. aureus* (N=31) and suspected hybrid forms (N=134) were collected from the commercial catch of Lake Edku at El-Meadia Fishery Centre from March 2006 to February 2007. The total length ranged between 11-21cm for *O. niloticus* specimens, 10-21cm for *O. aureus* specimens and 11-18 cm for the suspected hybrid forms.

Agar-Starch-Polyvinyl Pyrolidone (P.V.P.) gel electrophoresis was carried out according to the procedures described by Shaw and Kaen [14], El-Metainy *et al.* [15] and Sabrah and El-Metainy [16]. For electrophoretic esterase analysis, liver and kidney were taken from 64 Nile tilapia, 31 blue tilapia and 134 hybrid forms live specimens. According to the results of esterase electrophoretic patterns, gene frequency of alleles segregating at each locus and in combined organs tissues were estimated.

The maximum-likelihood is used to estimate the hybrid index. The likelihood function is determined by the unknown individual's genotype and the frequencies of alleles within each of the parental species at each of the

loci. It was calculated according to the procedures described by Rieseberg *et al.* [17]. The identity of genes and genetic distance were estimated between each species and type according to Nei [18].

RESULTS

In the present study, three hybrid forms of tilapia fish were morphologically identified. The Hybrid 1 (H1) is characterized by a straight head profile, pink or reddish dorsal fin edge and the caudal fin with a broad pink to bright red distal margin but unmarked by any vertical stripes. The hybrid 2 (H2) is distinguished by a straight or slightly convex head profile, reddish dorsal fin edge and caudal fin with a broad pink to bright red distal margin has one to three complete and few uncompleted dark vertical stripes. The hybrid 3 (H3) is characterized by a straight head profile, black dorsal fin edges and caudal fin with a broad black to bright red distal margin has one to three complete and few uncompleted vertical bars. Parental and hybrid specimens are shown in Fig. (1).

The electrophoretic pattern of esterase isozymes from liver of putative parents and the intermediate morphological forms showed four anodal and one cathodal loci. The same pattern was found for kidney except of *O. aureus* whereas, it revealed only three anodal and only one cathodal loci controlled these isozymes. Moreover, the locus Est-3A showed higher activities than other loci in all examined specimens.

Esterase isozymes genes were different in homozygosity or heterozygosity in the two putative species. In the liver of all examined groups, the loci Est-1A, Est-2A and Est-1C showed homozygous alleles, while

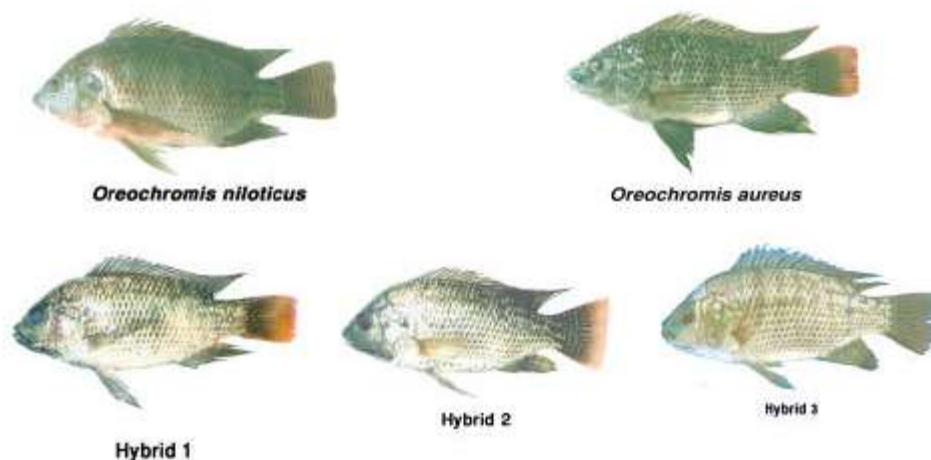


Fig. 1: Tilapia putative parents and their different hybrid forms

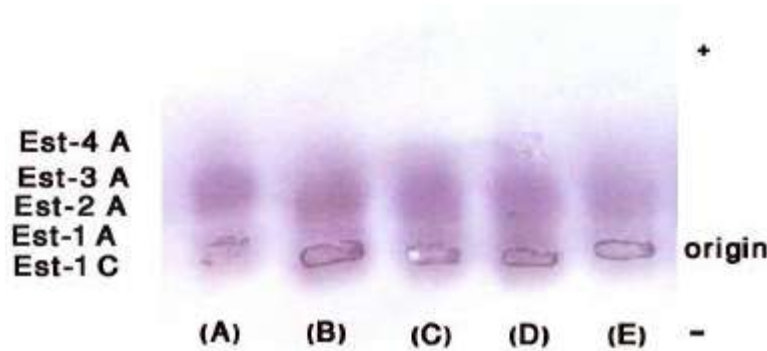


Fig. 2: Esterase zymogram extracts from liver of hybrid 1 (A), hybrid 2 (B) and hybrid 3 (C) and Putative parents Nile tilapia (D) and Blue tilapia (E).

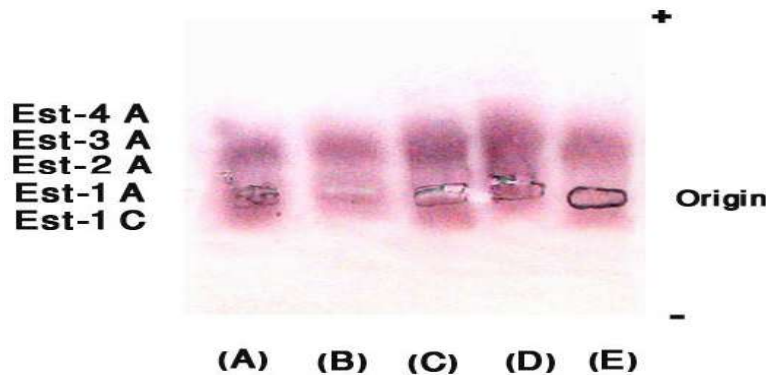


Fig. 3: Esterase zymogram extracts from kidney of hybrid 1 (A), hybrid 2 (B) and hybrid 3 (C) and Putative parents Nile tilapia (D) and Blue tilapia (E).

Table 1: Gene frequency estimates for alleles segregating at different loci coding for liver esterase isozymes of the hybrids and their putative parents, *O. niloticus* and *O. aureus*

Species	Est-4 A		Est-3 A		Est-2 A		Est-1 A		Est-1C	
	S	F	S	F	+	-	+	-	+	-
<i>O. niloticus</i>	0.683	0.317	0.533	0.467	0.833	0.167	0.767	0.233	0.833	0.167
<i>O. aureus</i>			0.500	0.500	0.200	0.800	0.867	0.133	0.667	0.333
Hybrid 1	0.577	0.423	0.462	0.538	0.538	0.462	0.769	0.231	0.846	0.154
Hybrid 2	0.581	0.419	0.514	0.486	0.595	0.405	0.784	0.216	0.784	0.216
Hybrid 3	0.594	0.406	0.531	0.469	0.375	0.625	0.688	0.312	0.875	0.125

Est-3A and Est-4A for Nile tilapia and hybrid forms and only Est-3A of blue tilapia had heterozygous alleles. While in kidney, the loci Est-3A and Est-1C in *O. niloticus* and hybrid specimens and Est-3A in *O. aureus* showed heterozygous alleles. The genes controlled these isozymes are five loci for all examined fish groups in both organs except *O. aureus*, which has four loci in the liver, whereas the locus Est-4A is completely absent from this organ (Fig. 2, 3).

Gene frequency of esterase isozymes for alleles segregating at different loci revealed that Est-3A was the

invariable expression in relation to organs and / or species. In liver of all examined fishes the Est-2A, Est-1A and Est-1C loci showed variable expression, while Est-4A was completely absent from liver of blue tilapia (Table 1). Data of esterase isozymes from kidney indicated that Est-3A revealed invariable expression, while other loci showed variable expression in kidney's tissue for examined fishes. Moreover, Est-1C was the only locus, which expressed as dominant in *O. aureus* and co-dominant in *O. niloticus* and hybrid specimens (Table 2).

Table 2: Gene frequency estimates for alleles segregating at different loci coding for kidney esterase isozymes of the hybrids and their putative parents, *O. niloticus* and *O. aureus*

Species	Est-4 A		Est-3 A		Est-2 A		Est-1 A		Est-1C	
	+	-	S	F	+	-	+	-	S	F
<i>O. niloticus</i>	0.357	0.643	0.536	0.464	0.821	0.179	0.679	0.321	0.571	0.429
Hybrid 1	0.200	0.800	0.600	0.400	0.700	0.300	0.900	0.100	0.450	0.550
Hybrid 2	0.514	0.486	0.514	0.486	0.703	0.297	0.713	0.287	0.622	0.378
Hybrid 3	0.563	0.437	0.563	0.438	0.625	0.375	0.563	0.437	0.781	0.219

Species	Est-4 A		Est-3 A		Est-2 A		Est-1 A		Est-1C	
	+	-	S	F	+	-	+	-	+	-
<i>O. aureus</i>	0.429	0.571	0.464	0.536	0.714	0.286	0.643	0.357	0.929	0.071

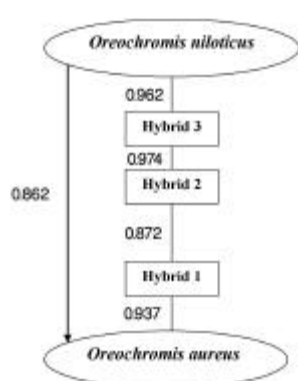


Fig. 4: Diagram of Nei's genetic identity between parental species and their hybrids captured from Lake Edku

Genetic identity (GI) and Nei's genetic distance (GD) between parental and hybrid specimens derived from combined organs are shown in Table (3). This table declares that the maximum genetic distance value (GD = 0.15) was found between parental species, while it was ranged from 0.14 to 0.03 in hybrid forms. The minimum value of genetic identity 0.86 was found between *O. niloticus* and *O. aureus*, while the maximum one (GI = 0.97) was found between hybrid 2 and hybrid 3. High magnitude of genetic identity estimate (GI = 0.96) was observed between *O. niloticus* and hybrid 3 and between *O. aureus* and hybrid 1 (GI = 0.94), while hybrid 2 showed intermediate identity with *O. niloticus* and *O. aureus* as shown in Fig. (4).

The hybrid index of hybrid 1 ranged from 0.00 to 0.81 with average value 0.44; this index revealed higher mean value (0.45) in hybrid 2 with range from 0.01 to 0.80. While hybrid 3 showed the same mean value of hybrid 2 and index range from 0.01 to 0.79.

Table 3: Estimates of Nei's genetic identity and genetic distance between pairs among the hybrids and its putative parents, *O. niloticus* and *O. aureus* based on different loci coding for esterase isozymes in combined organs

Species	<i>O. niloticus</i>	<i>O. aureus</i>	Hybrid 1	Hybrid 2	Hybrid 3
<i>O. niloticus</i>		(0.148)	(0.120)	(0.045)	(0.039)
<i>O. aureus</i>	0.862		(0.066)	(0.109)	(0.118)
Hybrid 1	0.887	0.937		(0.137)	(0.140)
Hybrid 2	0.956	0.897	0.872		(0.027)
Hybrid 3	0.962	0.889	0.870	0.974	

The genetic distance is given in parentheses

Using maximum-likelihood to estimate the hybrid index revealed that these hybrid forms (H1, H2 and H3) have 81%, 80% and 79% affinity with the parental species respectively.

DISCUSSION

Many of hybrids in nature are the results of interference by man's activities such as reservoir building, introduction of exotic species, modifications of rivers ect. [19]. Blue tilapia has an ability to tolerate salinity concentration higher than Nile tilapia, which prefers fresh and brackish waters. Therefore, blue tilapia is not only found in freshwater, but can also inhabit in brackish and saline waters [20]. It has been reported that blue tilapia has bred in marine waters of Tampa Bay, Florida [21]. In Lake Edku, blue tilapia inhabits near the Lake-Sea connection, while Nile tilapia inhabits in other parts of the Lake, especially in the areas near the drainage water discharge.

Lake Edku received during the last decades high rates of drainage water discharge, which usually takes its way, through the lake, to the sea decreasing the salinity of the

lake water, especially at the area near the Lake-Sea connection. The average value of salinity in this area was found to be around 21.5 parts per thousand since 20 years, this average decreased to 2.52 parts per thousand during the year 2000 [22]. Such decrease deleted the role of salinity as a geographical reproductive barrier. Moreover, both species have an overlapping spawning periods [23, 24] and display similar spawning behaviors (maternal brooder). All these reasons led to the occurrence of natural hybridization between these two species.

The existence of natural hybridization between *O. niloticus* and *O. aureus* in Lake Edku agrees with findings of [3], this author compared the suspected natural hybrid male from Lake Edku with artificial hybrids by the methods of biochemical characteristics of soluble muscle proteins and amino acids contents. The results revealed that this natural hybrid has a concentration of amino acid quite close to that of the experimental hybrid between the male of *O. niloticus* and female of *O. aureus*. In addition, the presence of five common protein bands at the same PI^s values confirmed that these natural hybrids resulted from crossing between these two mentioned species.

The use of molecular genetics is increasingly contributing to our knowledge of fundamental issues in evolutionary biology of aquatic organisms for determining the role of evolution forces involved in population divergence and, ultimately, speciation events [25-27]. However, the esterase isozymes as the active proteins have been extensively used as a genetic marker to separate populations and postulated sibling species and to study genetic variability among populations [28, 29]. So, in the present study, esterase isozymes were used to discover genetic patterns of examined fish groups to determine if the morphological intermediate fish forms resulted from hybridization.

In this investigation, the gene frequency and genetic distance were estimated for alleles segregated at different loci coding for esterase isozymes in liver and kidney, which revealed the highest activity units more than other organs in tilapia species. These findings agree with [13]. The specificity of isozymes to hybrid types and pure species was indicated by the Est-4A locus, which is absent in the liver of *O. aureus* and present in the liver of *O. niloticus* and other hybrid forms. In addition, the variation in number of esterase isozymes bands is higher between the parental species than between the hybrid forms. This difference in the number of esterase isozymes

bands, which has different epigenesis at the level of isozymes and allozymes, between the two species is in agreement with [12, 13]. These authors mentioned that the genes controlled these isozymes were more in *O. niloticus* than in *O. aureus*.

The genetic distance ($GD = 0.15$) between *O. niloticus* and *O. aureus* revealed the great genetic divergence between them. This finding is in accordance with that reported by Himeda [13]. Moreover, the genetic identity may be considered as a key parameter for summarizing the phylogenetic relationships between parental species and hybrid forms. In the present study, the intermediate genetic identity between the two parental species and the other hybrid forms confirmed the hybrid nature of these forms.

The hybrid index is a simple likelihood model, which is an estimate of the proportion of alleles that were inherited from one of the two parental species. The estimate of hybrid index is affected by the magnitude of allele frequency differences between parental species. It has been applied in several studies [17, 30-32]. The result of hybrid index showed high affinity between intermediate morphological forms and putative parents, *O. niloticus* and *O. aureus* indicating that these forms arose through hybridization between these reference species.

Hence, this electrophoretic study gives an evidence for the hybrid nature of intermediate morphological specimens, which resulted from crossing between *O. niloticus* and *O. aureus* in Lake Edku.

It can be concluded that, this electrophoretic study gives an evidence for the hybrid nature of the intermediate morphological specimens, which resulted from crossing between *O. niloticus* and *O. aureus* in Lake Edku.

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