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Genetic Structure of Natural Egyptian *Oreochromis niloticus* Evaluated Using Dominant DNA Markers

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Abstract: Genetic Markers revealed from randomly amplified polymorphic DNA-polymerase chain reaction analysis (RAPD-PCR) were used to estimate the gene flow, detect the genetic diversity and construct phylogenetic relationships of some Egyptian Nile Tilapia (*Oreochromis niloticus*) populations collected from three different locations (Aswan-Nasser lake, Giza and Quanater) For monitoring the changes in population structure and genetic variation of these populations. Nei's genetic distances among applied *O.niloticus* populations were calculated and found to be 0.1304, 0.1305 and 0.1487 between Aswan (As)-Giza (G), Aswan (As)-Quanater (Q) and Giza (G)-Quanater (Q) populations' pairs, respectively. The respective estimated gene flow (Nm) was 1.84, 1.611 and 1.536 with average of about 1.65. The averages of band frequencies generated by all primers were 0.26 for Giza and 0.23 for both Aswan and Quanater populations. In conclusion, we found a relatively high level of genetic diversity which is required for populations to be more adaptive with the environmental changes and that the estimated gene flow values were always more than 1 and approximately close to 2 and this indicates the presence of sufficient number of migrant among studied populations.

Key words: Oreochromis niloticus · gene flow · DNA markers conservation · microsatellite · genetic diversity · F-statistics

INTRODUCTION

It is important to study aquatic organism's biodiversity, especially fish because they are the only major human food source that is primarily harvested from wild populations [1]. Therefore, methods for the development of populations' management guidelines follow often more closely those commonly used for wildlife compared to domestic animals and plants

Several fish, especially family *Cichlidae* have become popular with aquaculturists.

Tilapias (family *Cichlidae*) have been introduced and transferred around the world from their endemic origins in Africa and Middle East [2]. According to Trewaves [3], Tilapia as a common name was divided into three main genera, *Tilapia*, *Sarotherodon* and *Oreochromis*. The *Oreochromis niloticus* species has become the most important one for freshwater aquaculture in tropics and subtropics because of its relative ease for culture and breeding in variety of aquaculture systems and the favorable attributes of these fish as food [4], the comparison of diversity within and among fish populations should reflect the ecological performance of *O. Niloticus* [5].

Genetic markers contribute in the conservation of species by aiding in detection of illegal hunting and by resolving important aspects of species biology. They have been used to detect selection and to estimate the effective population size. In addition, they have been used in determining parentage, sex, mating system, population structure and to detect introgression [6]. Several molecular techniques were applied to detect DNA markers and to reflect the genetic background of fish populations [7, 8].

In this study, RAPD markers were used to assess levels of gene flow and genetic polymorphisms among three local *O.niloticus* populations and briefly discuss the conservation and management implications of the findings.

MATERIALS AND METHODS

All laboratory works and data analysis were performed during the years 2006 and 2007. At the

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Table 1: Primer code, sequence and average of band frequencies generated by each primer across all *O.niloticus* populations

Primer code	Primer sequence	Average of band frequencies
OPA17	3'-GAC CGC TTG T-5'	0.229
OPA19	3'-CAA ACG TCG G-5'	0.161
OPA20	3'-GTT GCG ATC C-5'	0.142
OPB03	3'-CAT CCC CCT G-5'	0.233
OPB12	3'-CCT TGA CGC A-5'	0.283
OPC03	3'-GGG GGT CTT T-5'	0.260
OPC05	3'-GAT GAC CGC C-5'	0.329
OPC12	3'-TGT CAT CCC C-5'	0.246
OPC16	3'-CAC ACT CCA G-5'	0.228
OPC20	3'-ACT TCG CCA C-5'	0.314

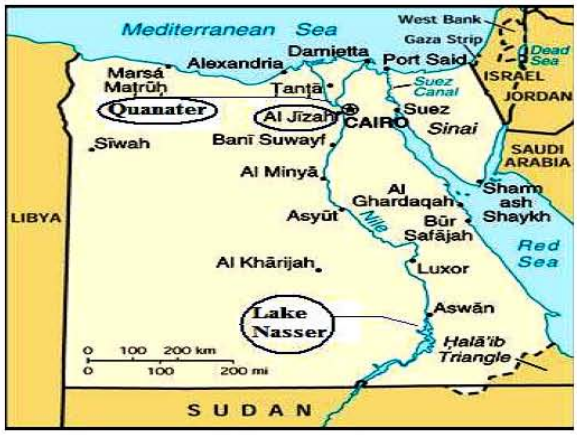

Fig. 1: Map of Egypt showing locations of *Oreochromis niloticus* populations. Studied locations are encircled

beginning of 2006, twenty *O.niloticus* individuals were sampled from each of three Nile River locations in Egypt, namely Lake Nasser (it locates near Aswan), Giza and Quanater (Fig. 1). From each specimen, approximately 1 x 1 cm) of caudal fin tissue was excised, placed in 70 % isopropanol and held at +4°C for subsequent DNA extraction. DNA extraction and purification were performed according to [9].

Ten primers (Operon Technologies, Inc.; Alameda, California, EUA) were initially screened for consistently reproducible and scoreable amplified bands (Table 1). PCR mixture was prepared according to the instructions provided with the Taq polymerase enzyme *(GoTaq® Flexi DNA polymerase; Catalog# M8301)* purchased from Promega Co.

The reaction conditions involved initial denaturation of DNA for 4 minutes at 94°C, 30 cycles of 45 sec denaturation at 94°C, 45 sec annealing at 37°C, 45 sec extension at 72° C and one 5 min cycle at 72° C for final extension. The amplification products were separated on 1.5% agarose gels stained with ethidium bromide, run in 1X TBE buffer at a constant voltage of 80 V.

Data analysis: The data were analyzed with *POPGENE (version 1.32)*, which is a Microsoft Windows-based freeware program for population genetic analysis [10]. The estimated parameters were: Polymorphic RAPD fragment (for convenience, it was treated as an allele) frequencies, allelic richness, proportion of polymorphic loci, heterozygosity, F-Statistics [11], genetic distance and identity [12]. Dendrogram was constructed based on Nei's genetic distances using UPGMA.

RESULTS

Intra-populations' genetic diversity: Eight parameters were used to assess the genetic variation within populations and they were: number of loci (bands), similarity, number and percentage of polymorphic loci, actual number of alleles (n_a), effective number of alleles (n_e), Nei's gene diversity (h) and Shannon's information index (I), expected heterozygosity (H_S) averaged across populations (intrapopulation diversity for subdivided populations).

With the ten examined primers, a total of 256 scoreable bands were produced. The number of bands (each band is considered as a dominant allele for the locus defined and designated by this allele) was variable among populations and ranged from 16 to 34. Band frequency averaged across all used primers for each population was 0.26 for G and 0.23 for both As and Q populations (Table 2). The average of allele frequencies ranged between 0.13 and 0.32 for As-population, between 0.16 and 0.34 for G-population and from 0.12 to 0.34 (Table 2).

The similarity values were 0.48, 0.53 and 0.69 for As, G and Q populations respectively (Table 2).

Results of the analyzed data showed that the percentage of polymorphic loci were 54.3, 60.1 and 49.04 % for As, G and Q populations respectively (Table 3).

As seen from Table 3, the average actual number of alleles across studied loci for each population (n_a) was 1.54, 1.6 and 1.49 for As, G and Q populations, respectively. While, the effective number of alleles across all loci for each population (n_e) was 1.31 for G-population as the highest value against 1.27 and 1.25 for As and Q populations, respectively.

The highest value of Nei's gene diversity (h = 0.19) and Shannon's Information index (I = 0.29) were observed in G-population. While, the lowest values were found in

Table 2: Average frequency of bands obtained using ten oligonucleotide primers with three *O.niloticus* populations collected from three deferent locations

	Primers											
Population	A17	A19	A20	B3	B12	C3	C5	C12	C16	C20	Av.	Simi.
(A)	0.20	0.13	0.16	0.27	0.28	0.23	0.32	0.24	0.20	0.32	0.23	0.48
(G)	0.23	0.24	0.16	0.19	0.34	0.27	0.32	0.29	0.26	0.33	0.26	0.53
(Q)	0.26	0.12	0.12	0.24	0.23	0.28	0.34	0.22	0.22	0.29	0.23	0.69

Table 3: The mean and standard deviation of Observed number of alleles (n_a), effective number of alleles (n_e), gene diversity (h), Shannon's Information index (I), Number of Polymorphic loci (NP) and the percentage of polymorphic loci (%NP) of the three studied population

	Population's parameters					
Populations	na±St.D	ne±St.D	h±St.D	I±St.D	NP	%NP
Aswan	1.54±0.49	1.27±0.32	0.17±0.18	0.26±0.27	113	54.3
Giza	1.6±0.49	1.31±0.32	0.19±0.18	0.29±0.27	125	60.1
Quanater	1.49±0.50	1.25±0.32	0.16±0.19	0.23±0.27	102	49.04

Table 4: Mean genetic differentiation (G_{ST}), gene flow (Nm), total heterozygosity (H_T) and within population heterozygosity (H_S) based on RAPD dominant markers for each pair of populations

	H_T	H_S	G_{ST}	X2	df	Nm
As/G	0.23	0.18	0.21	17.04	1	1.84
As/Q	0.22	0.17	0.24	18.94	1	1.61
G/Q	0.23	0.18	0.25	19.64	1	1.54
average	0.23	0.18	0.23	27.816	2	1.65
Total	0.25	0.17	0.29			1.24

Q-population (h = 0.16 and I = 0.23). However, As population had in between values which were 0.17 and 0.26 for gene diversity (*h*) and Shannon's Information index (I) respectively. The intrapopulation diversity values (H_S) ranged between 0.17 and 0.18 with average of 0.18 and the intrapopulation diversity for overall populations was 0.17 (Table 4).

Inter-populations' genetic diversity: Five parameters were calculated to investigate the Genetic variation among populations. These parameters are: expected heterozygosity (H_T) for the total populations (total diversity), the genetic differentiation (G_{ST}), gene flow (Nm), genetic distance (D) and genetic identity.

The total diversity values (H_T) ranged between 0.22 and 0.23 with an average of 0.23 and the overall populations estimated total diversity was 0.25.

Genetic differentiation (G_{ST}) across all loci between each pair of populations were 0.21, 0.24 and 0.25 for As-G, As-Q and G-Q populations' pairs respectively with an average of 0.23 and the overall G_{ST} value was 0.29 (Table 4).

Table 5: Nei's genetic Identity (above diagonal) and Nei's genetic distance (below diagonal)

populations	Aswan	Giza	Quanater
Aswan		0.88	0.88
Giza	0.130		0.86
Quanater	0.131	0.15	

Table 6: lengths among taxa (populations) and nodes on the phylogenetic tree

Between	And	Length
2	1	0.46
1	A	6.52
1	G	6.52
2	Q	6.98

The estimated gene flow (Nm) values were 1.84, 1.61 and 1.54 for As-G, As-Q and G-Q populations' pairs respectively with an average of 1.65 While the overall gene flow was 1.24 (Table 4).

Genetic distances (D) among applied *O. niloticus* populations were calculated according to Nei [13] and they were 0.13, 0.131 and 0.15 between As-G, As-Q and G-Q population's pairs respectively. The genetic identity values were 0.88, 0.88 and 0.86 between the same pairs of populations (As-G, As-Q and G-Q) as shown in Table 5.

The dendrogram presented in Fig. 2 shows the genetic relationships among the applied fish populations based on RAPD data. The lengths among sites on Figure (2) were 0.46 and 6.98 between (2, 1) and (2, Q) respectively. Also, they were the same (6.52) between both of (1, As) and (1, G) as presented in Table 6.

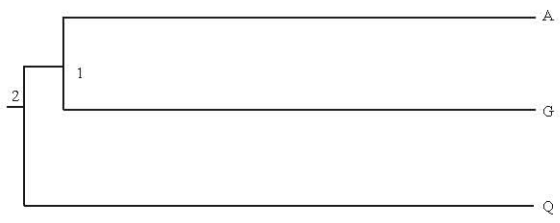

Fig. 2: Dendrogram represent the inferred phylogenetic relationships among relevant populations

DISCUSSION

The ten examined primers produced a total of 256 scoreable bands (each band is considered as a dominant allele for the locus defined and designated by this allele) where the number of bands was variable among populations and ranged from 16 to 34 as an initial indicator for the existed polymorphism.

Comparing actual allele number (n_a) across studied loci for each population (the highest value was 1.6) and the effective number (n_e) of alleles (the highest value was 1.31) showed that the first approximately reaches two while the second was more close to one. Number of alleles was used as a measure of genetic polymorphism [14] and it varied among the present studied populations as we observed.

It was found that the estimated allele frequencies varied among populations. These values reflect low levels of similarity within each population. The similarity was estimated and in fact, was found to be at low levels indicating presence of high genetic variation in the studied populations. Analyzed data showed a relatively high percentage of polymorphic loci that were 54.3, 60.1 and 49.04 % for As, G and Q populations, respectively reflecting noticeable levels of genetic polymorphisms in the studied populations

Gene diversity (h) and Shannon's Information index (I) were clearly different from zero and reflect presence of relatively high genetic diversity level. It is known that large populations of naturally outbreeding species usually have extensive genetic diversity [15]. But, because of dominance behavior of RAPD markers they can not provide totally reliable estimates of heterozygosity [16, 17], or direct interpretation of allele frequencies [18], without making several assumptions.

All intra-population diversity (H_S) and inter-population diversity (H_T) values were positive and clearly different from zero indicating the existence of heterozygote deficiency. Average genetic differentiation (G_{ST}) across all loci was 0.23 and the overall G_{ST} value was 0.29. All these values were statistically significant indicating existence of high levels of genetic divergence (P = 0.005). But, no significant differences were detected between these values for different populations' pairs. Also, the estimated G_{ST} values should be interpreted with caution, as they are a useful relative measure of variation between populations [19].

In this study, the estimated gene flow (Nm) values were greater than 1 and approximately equal 2 indicating that the number of immigrants between these populations' pairs per generation is two individuals and is highly sufficient to counteract the effects of genetic drift and inbreeding under natural conditions [20]. Although it is known that the genetic differentiation generally increases with increasing geographical isolation [21], the present results did not show the same trend. Gene flow between A and G populations, was higher than that existed between A and Q populations and this logically agrees with the geographical distance. But, There is a small difference between the Nm values for As and Q populations' pair (1.65) and that for G and Q populations' pair (1.54) and this is not consistent with the geographical distances between each pair. The high estimated gene flow reduced population fragmentation factors and was sufficient to counteract population differentiation (G_{ST}).

All applied populations were collected from the same river and there is a human effect on the genetic equilibrium due to fish transportation from location to another without restricted plan. So, it is likely that genetic equilibrium has not been reached and thus, over-estimates of gene flow may be existed.

Genetic distances (D) among applied *O.niloticus* populations were 0.13, 0.131 and 0.15 between As-G, As-Q and G-Q population's pairs, respectively. The first two values are very close and smaller than the third indicating that the largest genetic distance between G and Q is in contradiction with the geographical distance on the land. Genetic identity values have the same trend as genetic distance among populations' pairs.

The dendrogram shows that the Q population is more distant from the other two populations (As and G). The two later populations were close to each other. We can suggest that the reason for the low RAPD based distances among the applied fish populations is due to a high gene flow values among studied populations.

Construction of High dame may have been preventing the migration (subsequently gene flow) from southern regions towards the others preventing the southern *O. niloticus* fish from the movement to north. But, this was not the observed reality and the differences

from expectations may be due to permitting *O. niloticus* cage culture in the Nile River or as a result of fish transfer by farmers when they move some of this fish from southern to northern regions (personal note).

In conclusion, this study revealed a relatively high level of gene flow and genetic diversity which is required for populations to be more adaptive with the environmental changes.

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