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Inferring the Genetic Homogeneity and Polymorphism in Some *Oreochromis niloticus* Broodstocks Using Molecular DNA Markers

[1]Mariam G. Eshak, [2]Y.M. Saad and [3]M.A. Rashed

[1]Department of Cell Biology, National Research Center, Dokki, Giza, Egypt
[2]Genetics Lab. NIOF, Alexandria, Egypt
[3]Department of Genetics, Faculty of Agriculture,
Ain Shams University, Shubra El-Khema, Cairo, Egypt

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Abstract: RAPD-PCR was used for inferring the genetic homogeneity and polymorphism in three farmed *Oreochromis niloticus* broodstocks (Abassa; Ab, Quanater; Q and Serow; S). The averages of band frequencies generated by all tested primers were 0.85, 0.72 and 0.68 for Ab, Q and S populations respectively. Results of the analyzed data showed that the number of polymorphic loci and percentage of polymorphic loci were 44 (19.2%), 76 (33.1%) and 60 (26.2%) for Ab, Q and S populations respectively. The genetic distance values were 0.41, 0.45 and 0.19 between Ab-Q, Ab-S and S-Q population's pairs respectively. The genetic identity values were 0.66, 0.64 and 0.83 between the same pairs of populations (Ab-Q, Ab-S and S-Q). The reason for the high RAPD based distances between Ab and both Q and S populations is due to location isolation between Ab and both S and Q populations.

Key words: *Oreochromis niloticus* · DNA markers · Microsatellite · Genetic diversity

INTRODUCTION

Aquaculture of *Oreochromis niloticus* output needs to increase several folds in order to meet the rising demands for this economic fish in Egypt in the future. Molecular biology including DNA markers [1] as marker assisted selection [2] can provide the means to increase the intensity and capacity of the operation.

Several molecular techniques were applied to detect DNA markers and to reflect the genetic background of fish populations [3, 4]. Molecular markers have been used to detect: bottlenecks, inbreeding, effective size, selection, parentage, sex, mating system, population structure, dispersal rates, population sizes, diet, disease status and introgression [5].

RAPD markers were widely used to detect the homogeneity and polymorphism within and among fish species and subspecies [1, 6]. In addition, this technique was used to estimate the genetic effect of mixed cultured fish with wild populations. Such DNA markers [7] could also be used to assess the impact of international or accidental release of farmed fishes to the wild [8].

The purity guarantee of Tilapia species such as *Oreochromis niloticus* is necessary for hybridization purpose. Research is desperately needed to improve production traits such as growth rate, feed efficiency, product quality and reduce disease susceptibility and the environmental impact of production systems [9]. This will require research efforts in reproduction, nutrition, genetics, growth development, immunology, water quality and production systems.

The present work aims to infer the genetic homogeneity and polymorphism in some *Oreochromis niloticus* broodstocks using molecular DNA markers.

MATERIALS AND METHODS

Sampling and DNA Extraction: Ten *O. niloticus* individuals were sampled from each of three Egyptian fish farms namely Abassa (Ministry of agriculture), Quanater National Institute of Oceanography and Fisheries (NIOF) and Serow (NIOF). From each specimen, approximately (0.5 cm x 0.5 cm) of caudal fin tissue was excised, placed in 70 % Ethanol, at 4°C for subsequent DNA extraction.

Corresponding Author: Mariam G. Eshak, Department of Cell Biology, National Research Center, Postal code: 12622, Dokki, Giza, Egypt.

Table 1: Primer code and their sequences.

Primer code	Primer sequence
OPA17	3'-GAC CGC TTG T-5'
OPA19	3'-CAA ACG TCG G-5'
OPA20	3'-GTT GCG ATC C-5'
OPB03	3'-CAT CCC CCT G-5'
OPB12	3'-CCT TGA CGC A-5'
OPC03	3'-GGG GGT CTT T-5'
OPC05	3'-GAT GAC CGC C-5'
OPC12	3'-TGT CAT CCC C-5'
OPC16	3'-CAC ACT CCA G-5'
OPC20	3'-ACT TCG CCA C-5'

DNA extraction and purification were performed according to Hillis *et al*. [10] method with some modifications (as described by Rashed *et al.* [7].

RAPD-PCR: Ten primers (Operon Technologies, Inc.; Alameda, California, USA) were initially screened for consistently reproducible and scorable amplified bands. These primers were: OPA17, OPA19, OPA20, OPB03, OPB12, OPC03, OPC05, OPC12, OPC16 and OPC20 (Table 1). PCR mixture was prepared according to the pamphlet provided with the Taq polymerase enzyme (GoTaq® Flexi DNA polymerase; Catalog# M8301) purchased from Promega Corporation distributer.

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 gel stained with ethidium bromide, run in 1X TAE 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 [11].

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 [12], genetic distance and identity [13]. Dendrogram was constructed based on Nei's genetic distances using UPGMA.

RESULTS

Intra-populations Genetic Diversity: Nine parameters were used to assess the genetic variation within studied fish populations: number of loci (bands), band frequencies, homogeneity, 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).

With the nine examined primers, a total of 229 scorable bands were produced with molecular size ranged from 10 to 2460 bp. The bands frequencies obtained using the ten RAPD primers with the three *O.niloticus* populations were calculated and presented in Table (2). The average band frequencies ranged between 0.66 and 1 with an average of 0.851 for the Ab-population, between 0.48 and 0.9 with an average of 0.72 for the Q-population and from 0.54 to 0.85 with average of 0.67 for S-population (Table 2).

Results of the analyzed data showed that the number of polymorphic loci and percentage of polymorphic loci were 44 (19.2%), 76 (33.1%) and 60 (26.2%) for Ab, Q and

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

	Primers											
Populations	A17	A19	A20	B3	B12	C3	C5	C12	C16	C20	AV.	SD
μ(Ab)	0.68	0.77	0.66	0.93	0.91	0.91	1	0.82	0.93	0.9	0.85	0.11415
SD(Ab)	0.3	0.3	0.3	0.1	0.05	0.11	0	0.2	0.16	0.2		
μ(Q)	0.5	0.48	0.88	0.645	0.8	0.76	0.56	0.90	0.9	0.79	0.72	0.16347
SD(Q)	0.3	0.25	0.21	0.28	0.21	0.26	0.23	0	0.22	0.24		
μ(S)	0.54	0.55	0.84	0.71	0.56	0.73	0.68	0.63	0.85	0.70	0.68	0.1114
SD(S)	0.29	0.38	0.35	0.34	0.35	0.3	0.33	0.47	0.3	0.22		

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 populations.

	Population's parameters					
Populations	na ± SE	ne ± SE	h ± SE	I ± SE	NP	%NP
Ab	1.19 ± 0.12	1.11 ± 0.08	0.07 ± 0.04	0.10 ± 0.06	44	19.2
Q	1.33 ± 0.14	1.2 ± 0.1	0.12 ± 0.05	0.18 ± 0.08	76	33.1
S	1.26 ± 0.13	1.15 ± 0.09	0.09 ± 0.05	0.13 ± 0.07	60	26.2
All pop.	1.98 ± 0.02	1.4 ± 0.04	0.27 ± 0.02	0.43 ± 0.02		

Table 4: Nei's genetic identity (above diagonal) and Nei's genetic distance (below diagonal).

Populations	Ab	Q	S
Ab	0.91±0.02	0.66	0.64
Q	0.41	0.76±0.06	0.83
S	0.45	0.19	0.80±0.03

Table 5: Lengths among taxa (populations) and nodes on the phylogenetic tree.

Between	And	Length
2	Ab	21.30
2	1	11.8
1	Q	9.44
1	S	9.44

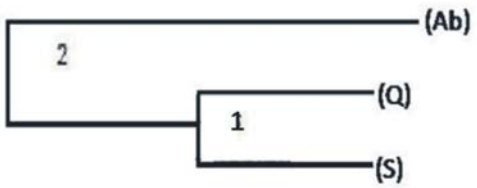

Fig. 1: Dendrogram represents the inferred phylogenetic relationships among the studied populations.

S populations respectively. Table (3) indicates that the average observed number of alleles across studied loci per each population (n_a) was 1.19, 1.33 and 1.26 for Ab, Q and S populations respectively. While, the effective number of alleles across all loci per each population (n_e) was 1.11, 1.2 and 1.26 for Ab, Q and S populations respectively (Table 3). The (Q) population had the highest 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) were detected in Table 3.

Inter-Populations Genetic Diversity:

Genetic distances (D) and genetic identity among the studied *O. niloticus* fish populations were calculated according to Nei [13] and presented in Table (4). The genetic distance values were 0.41, 0.45 and 0.19 between Ab-Q, Ab-S and S-Q population's pairs respectively. The genetic identity values were 0.66, 0.64 and 0.83 between the same pairs of populations (Ab-Q, Ab-S and S-Q) as presented in Table (4). The similarity values, within populations were 0.91, 0.76 and 0.80 for Ab, Q and S populations respectively (Table 4).

The dendrogram presented in Figure (1) shows the genetic relationships among the studied fish populations based on RAPD data. The lengths among sites were 21.30 and 11.8 between (2, Ab) and (2, 1) respectively. Also, they were the same (9.44) between both of (1, Q) and (1, S) as presented in Table (5).

DISCUSSION

Ten RAPD primers were tested by Rashed *et al.* [7] (which were used in the present study) to analyze intra and inter-populations genomic polymorphism in three wild *O. niloticus* populations. They obtained a significantly great number of PCR-amplified fragments (256 bands). Analysis of these fragments polymorphism enabled them to estimate the genetic diversity within and among those fish populations. So these 10 RAPD primers were chosen to study the genetic structures of the three farmed *O. niloticus* populations (Ab, Q and S).

In the present study, the results were analyzed by POPGENE as a specific software program for population genetic analysis. RAPD data analysis using this program was recommended to study the genetic polymorphism in tilapia populations and/or species by many authors such as [1, 6 and 7]. POPGENE was used by Saad [6] for analysis of genetic variations in the two *S. galilaeus* sexes to facilitate identification of the diversity within and between both of them.

The greatest advantages for RAPD technique are that it can potentially sample a large number of loci and that no prior DNA sequence information is needed to perform the assay [14].

In the present study, the ten examined primers produced a total of 229 scorable 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 studied fish populations (Ab, Q and S) as an initial indicator for the existed polymorphism.

Results from the analyzed data [7] showed that the percentage of polymorphic loci were 54.3, 60.1 and 49.04 % for Aswan, Giza and Quanateir populations respectively. In the present study, the highest polymorphic loci percentage was that of the (Q) population (33.1%) while the lowest percentage was detected in (Ab) population. Our results reflected noticeable levels of genetic polymorphism in the studied fish populations.

Comparing actual allele number (n_a) across studied loci for each population and the effective number (n_e) of alleles, it was observed that the highest numbers were those of the (Q) population. On the other hand, the lowest (n_a) and (n_e) values were those of the (Ab) population.

Number of alleles was used as a measure of genetic polymorphism [7, 15] and it varied among the present studied populations.

In the present study, the estimated allele frequencies varied among populations. These values reflect the levels of similarity within each population. The genetic similarity values were 0.91, 0.76 and 0.80 within the Ab, Q and S populations respectively. From these data, the highest genetic polymorphism was detected in the farmed (Q) population. While the results of Rashed *et al.* [7] showed lower similarity (0.48, 0.53 and 0.69) for the As, G and Q populations respectively, indicating the presence of high genetic variation in those studied fish populations. The genetic diversity, which is required for populations to be more adaptive with the environmental changes, can be measured using an array of molecular and quantitative methods.

In the present study, gene diversity (h) and Shannon's Information index (I) of the farmed *O. niloticus* populations were clearly different from zero and reflect presence of relatively genetic diversity level. The same conclusion was obtained by Rashed *et al*. [7] in the studied wild *O. niloticus* populations. They confirmed that large populations of naturally outbreeding species usually have extensive genetic diversity as described by Nei and Kumer [16].

In the present study, the highest percentage of polymorphic bands (33.1%) was detected in farmed (Q) population. On the other hand, this value was 49.04% in the wild (Q) population as reported by Rashed *et al*. [7]. The loss of genetic diversity is often associated with reduced reproductive fitness [5].

It was found that the percentage of polymorphic bands was relatively high [7], but because of dominance, RAPD cannot provide totally reliable estimates of heterozygosity [17] without making several assumptions. The studied populations were collected from different Egyptian fish farms. So, there is a human effect on the genetic homogeneity and equilibrium due to inbreeding and/or fish transportation from farm to another without restricted plan.

Genetic distances (D) among the studied *O. niloticus* populations were 0.41, 0.45 and 0.19 between Ab-Q, Ab-S and S-Q population's pairs respectively. The first two values are very close and differed from the third. This indicates that, there is a large genetic distance between the Ab and both the Q and the S populations. In addition, the low genetic distance between Q and S may be due to unregulated fish transportation from S farm to Q farm in the past, especially because both the Q and S farms belong to the same institute (NIOF, Egypt).

Genetic identity values were 0.66, 0.64 and 0.83 between the Ab-Q, Ab-S and S-Q respectively. These values have the same trend as the genetic distance among these populations' pairs.

The dendrogram presented in Figure (1) shows the genetic relationships among the applied fish populations based on RAPD data. From this Figure, it appears that the Ab population is more distant from the other two populations (S and Q). The two later populations were close to each other.

In conclusion, the reason for the high RAPD based distances between Ab and both Q and S populations may be due to location isolation between the Ab and both S and Q populations. In addition, the high calculated homogeneity value in the Ab population might be due to inbreeding. More DNA markers will be needed to screen over more Loci to obtain high accuracy of genetic variation estimates. An innovative breeding program parallel with molecular markers (as a marker assisted selection) is needed to select promising fish individuals that have economic characters as parents in the future.

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