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Analysis of Genetic Variations in Two *Sarotherodon galilaeus* Sexes Using Popgene

Y.M. Saad

Genetic Lab., National Institute of Oceanography and Fisheries, Egypt

Abstract: Genetic variations (revealed from RAPD markers) between the two sexes of *S. galilaeus* were analyzed using POPGENE (version 1.32) to spot light on the genetic diversity within and between both of them. Ten RAPD primers were tested on 20 individuals of each *S.galilaeus* sex separately.Results showed that the highest mean value of gene diversity (h = 0.35) and Shannon's Information index (I = 0.52) were observed in investigated males compared to females. The average of actual number of alleles (na) across studied loci was 1.96 for both two sexes.

Key words: *S. galilaeus* · Diversity · Population genetics

INTRODUCTION

In aquaculture, biotechnology including DNA markers and bioinformatics represents a range of technologies that present opportunities to improve management and conservation of wild stocks [1, 2 and 3]. Many computer programs now available for population genetic analysis. Such programs, present some information about the population structure, diversity and differentiation. POPGENE [4] is a user-friendly Microsoft Window-based computer package for the analysis of genetic variation among and within populations using co-dominant, dominant markers and quantitative traits. This package provides the Windows graphical user interface that makes population genetics analysis more accessible for the casual computer user and more convenient for the experienced computer user.

Tilapia fish were divided into three main genera [5]; *Tilapia, Sarotherodon and Oreochromis*. Tilapias are widely distributed in the river Nile and most fresh water fish farms. Within these fish, *S. galilaeus* (as genetic resources) needs more and more molecular studies for conservation. Conservation (documentation, characterization, evaluation and utilization of genetic resources) [6] needs of fishes are special in many respects compared to those of terrestrial organisms.

This work aims to outline some of the main POPGENE program applications for molecular data revealed from RAPD markers analysis in the two *Sarotherodon galilaeus* sexes to spot light on the genetic polymorphism within and between both of them.

MATERIALS AND METHODS

Samples were collected during March (2008) from four Egyptian locations namely Aswan (Lake Nasser = As), Quanatair (Q), Lake Manzala (M) and Abbasa (Sharkia = Ab).

DNA extraction and purification were performed according to [7].

Ten primers (Operon Technologies, Inc.; Alameda, California, EUA) were screened for scoreable amplified bands (Table 1). The primers were tested on 20 individuals of each *S. galilaeus* sex (20 males and 20 females) separately. PCR mixture preparation, reaction conditions and separation of PCR products were carried out as explained in [3].The data were analyzed with POPGENE (version 1.32) which is a Microsoft Window-based computer package for the analysis of genetic variations among and within populations [4].

The estimated parameters were: Polymorphic RAPD fragment (for convenience, it was treated as an allele) frequencies, proportion of polymorphic loci, heterozygosity, F-Statistics, genetic distance and Dendrogram (constructed based on Nei's genetic distances using UPGMA).

Table 1: RAPD Primers and their sequences used in the study

Code	Sequence	Code	Sequence
OPA7	3'- GAA ACG GGT G -5'	OPC07	3'- GTC CCG ACG A -5'
OPA10	3'- GTG ATC GCA G -5'	OPC08	3'- TGG ACC GGT G -5'
OPA18	3'- AGG TGA CCG T -5'	OPC09	3'- CTC ACC GTC C -5'
OPB13	3'- TTC CCC CGC T -5'	OPC17	3'- TTC CCC CCA G -5'
OPC03	3'- GGG GGT CTT T -5'	OPC18	3'- TGA GTG GGT G -5'

Corresponding Author: Y.M. Saad, Genetic Lab., National Institute of Oceanography and Fisheries, Egypt
E-mail: yasser_saad19@yahoo.com

RESULTS

A total of 151 RAPD bands were examined in a total number of 40 fish individuals (20 males and 20 females).

The percentages of polymorphic bands were 96.6% and 96% in males and females respectively.

Genetic variation statistics for all loci within each *S. galilaeus* sex: Between males, the highest variation values were detected in (M) while the lowest values were detected in (Ab) compared to others. The percentage of polymorphic loci (P %), (I), (h), (ne) and (na) in males were higher than females except in (Ab) population (Table 2).The highest value of (h = 0.35) and (I = 0.52) were observed in Males. The average of (na) across studied loci was 1.96 for both tow sexes (Table 3).

Out of 10 primers used, one primer (OPC07) matching a male specific DNA marker at MW a round 680 bp in all studied samples.

Genetic diversity for all loci between the two *S. galilaeus* sexes: The expected heterozygosity (HT) for total diversity, genetic differentiation (Gst), genetic distance (D) and genetic identity were calculated to investigate the genetic diversity between the applied two sexes. The mean of (HT) were 0.36 and 0.35 in males and females, respectively. The overall populations estimated total diversity was (0.357). Genetic differentiation (Gst) across all loci (Table 3) among all females (0.59) was higher than males (0.52).

Genetic distances (D) and identity among applied population within each sex were calculated and presented in Table 4. The highest (D) value was detected between

Table 2: Mean±SE of actual number of alleles (na), effective number of alleles (ne), Nei's gene diversity (h), Shannon's information index (I), percentage of polymorphic loci and number of polymorphic loci within each applied sex

	Population											
	Ab			Q			M			As		
Sex	♂	♀	Total	♂	♀	Total	♂	♀	Total	♂	♀	Total
na	1.33±0.08	1.4±0.08	1.5±0.08	1.5±0.1	1.39±0.08	1.64±0.08	1.5±0.08	1.4±0.08	1.6±0.08	1.35±0.02	1.28±0.08	1.43±0.08
ne	1.22±0.06	1.29±0.06	1.32±0.06	1.36±0.06	1.26±0.06	1.39±0.06	1.4±0.08	1.2±0.06	1.4±0.06	1.24±0.06	1.19±0.06	1.31±0.06
h	0.13±0.02	0.162±0.02	0.20±0.04	0.20±0.08	0.14±0.04	0.22±0.04	0.22±0.04	0.163±0.04	0.26±0.04	0.138±0.04	0.11±0.02	0.17±0.04
I	0.19±0.2	0.23±0.2	0.30±0.2	0.29±0.3	0.22±0.2	0.34±0.2	0.33±0.3	0.24±0.2	0.38±0.2	0.2±0.2	0.16±0.2	0.25±0.2
NPL	50	65	82	79	60	98	88	65	104	53	43	66
%PL	33.1	43.5	54.30	52.3	39.74	64.9	58.28	43.0 5	68.87	35.1	28.48	43.71

Pop = Population, Ab=Abassa, Q= Qanatair, M= Manzala, As= Aswan, ♂ = Male and ♀ = Female

Table 3: Mean and SD of actual number of alleles (na), effective number of alleles (ne), Nei's gene diversity (h), Shannon's information index (I), expected heterozygosity (HT), the genetic differentiation (Gst) and gene flow (Nm) for each applied fish sex across all studied populations

	na	ne	h	I	%PL	HT	Hs	Gst	Nm
♂	1.96±0.17	1.61±0.31	0.35±0.13	0.52±0.17	96.69	0.36±0.01	0.17±0.01	0.52	0.457
♀	1.96±0.1	1.59±0.3	0.34±0.1	0.51±0.1	96.03	0.35±0.02	0.14±0.01	0.59	0.34
Total	1.99±0.08	1.65±0.3	0.37±0.13	0.54±0.17	99.34	0.357±0.01	0.34±0.01	0.023	21.13

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

	Population											
	Ab			Q			M			As		
	Sex	♂	♀	(t)	♂	♀	(t)	♂	♀	(t)	♂	♀	(t)
Ab	♂	1			0.65			0.69			0.66		
	♀	….	1			0.64			0.69			0.65	
	(t)	---	---	1			0.70			0.76			0.70
Q	♂	0.41			1			0.74			0.65		
	♀		0.43		….	1			0.71			0.62	
	(t)	---	---	0.35	---	---	1			0.79			0.68
M	♂	0.36			0.29			1			0.74		
	♀		0.35			0.34		….	1			0.69	
	(t)	---	---	0.26	---	---	0.22	---	---	1			0.76
As	♂	0.41			0.42			0.29			1		
	♀		0.42			0.47			0.37		….	1	
	(t)	---	---	0.35	---	---	0.37	---	---	0.26	---	---	1

Pop = Population, Ab=Abassa, Q= Qanatair, M= Manzala, As= Aswan, ♂ = Male, ♀ = Female and t = Total

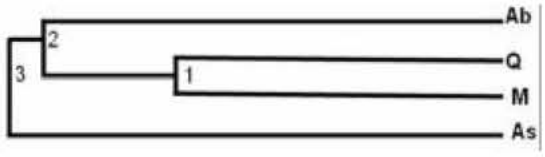

Fig. 1: Dendrogram represent the inferred phylogenetic relationships among applied fish populations. Ab=Abassa, Q= Qanatair, M= Manzala and As= Aswan. The lengths among sites were 1.18, 15.4, 4.1, 11.3, 11.3 and 16.6 between (3, 2), (2, Ab), (2, 1), (1, Q),(1, M) and (3, As) respectively

(Q & As) while the lowest value was detected between (Q & M) within each sex.

The dendrogram (Fig. 1) reflects the genetic distance among the applied fish populations. Q and As are distantly from each others while Q and M are the most related populations to each others. The same distance value between Ab &Q was calculated between Ab & As.

DISCUSSION

The greatest advantages for RAPD technique are that it can potentially sample a large number of loci and that no a prior DNA sequence information is needed to perform the assay [8]. In the present study, the analysis of RAPD data using POPGENE succeeded in screening the biodiversity among and within the applied *S. galilaeus* populations. 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 [9]. Therefore, methods for the development of populations' management guidelines follow often more closely those commonly used for wildlife compared to domestic animals, fish and plants. Comparison of amplification of DNA samples from the four genotypic sexes failed to show any reproducible and clear cut RAPD markers occurring in one sex alone accept one primer (OPC07). The DNA marker (Around 680 bp) generated by this primer could be used in molecular sexing in *S. galilaeus*. The most recent approach to studying the mechanism of sex determination in fishes is to develop sex-specific molecular markers. Sex-specific markers have been developed in the *Poecilia reticulata* [10], *O. kisutch* [11] and *Leporinus elongatus* [12].

The highest value of Nei's gene diversity and Shannon's Information index were observed in males while, the lowest values were found in females. On the other hand, genetic differentiation (Gst) across all loci among all females was higher than males. This due to that, the gene flow between investigated males is higher than females. This relatively high gene flow among males may be due to males transportation to introduce a new blood from the river Nile to Abassa farm (human effect) or for fish migration along the river Nile as between As to Q.

In this study, highest polymorphism was detected in Manzala (M) population. In addition, due to high genetic variations within both males and females within (M) the selection will be effective using a suitable breeding program parallel with molecular markers (as a marker assisted selection) to select promising individuals have some economic characters. Manzala Lake (large, shallow and brackish water) is one of the Deltaic Mediterranean lakes in Egypt. This lake is exposed to different levels of pollutants [13]. These pollutant sources may be work as selection factors for fish biotypes against other biotypes from the same species [14].

The relationship among applied fish populations was reconstructed to screen the genetic distance among applied fish populations.

One of the important points arising from the present study was to analyze the genetic variation between the two sexes of *S.galilaeus*. This confirmed by [15]. They reported that, sex determination and differentiation are fundamental components of the genetic information passed on from generation to generation.

It was concluded that, the analysis of genetic variations in the two *S.galilaeus* sexes using POPGENE facilitate identification of the diversity within and between both of them. Maintenance of genetic diversity is a primary objective in the management of wiled and captive fish populations. The selection will be effective using a suitable breeding program parallel with molecular markers (as a marker assisted selection) to select promising individuals have some economic characters from (M) population.

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