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GENETIC RELATIONSHIPS WITHIN AND AMONG FIVE EGYPTIAN *OREOCHROMIS NILOTICUS* POPULATIONS VIA BIOCHEMICAL AND MOLECULAR MARKERS

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ABSTRACT

Five Egyptian *Oreochromis niloticus* populations namely; El-Qanater, Kafr El-Sheikh, El-Serow (which represented three different farms), Aswan and Naser Lake were used to determine the ability of some biochemical [proteins (water and alcohol soluble proteins) and isozymes (Est, Adh & Mdh)] and molecular [A06, A07, A10, A19, A20, B08, C03, C05, C12 and C13 RAPD primers] markers for detecting the genetic variations and relationships within these populations. Ten individuals from each population were sampled and dissected. Biochemical and RAPD marker profiles recorded about 45.92% and 61.64% of polymorphism, respectively, with an average of 53.78% which indicated a moderate genetic variation within each of these populations. In addition, the genetic similarity averages within all tested populations based on biochemical and RAPD markers were 0.984 and 0.801, respectively, with an average of 0.893 which indicated that RAPD markers were more prominent for illustrating the genetic structure and detecting the degree of homogeneity and inbreeding within each of the tested populations.

On the other hand, the same ten RAPD primers were used to detect the genetic variations and relationships among these five tested populations against bulked DNAs of the ten individuals of each population. The ten used primers showed different levels of polymorphism percentages, which ranged from 100.00% using A06 primer to 16.67% using C05 primer among these five populations with an average of 66.67%. Moreover, the similarity matrix and constructed dendrogram based on the overall RAPD primers classified the five populations into two main clusters. Within the first cluster,

Naser Lake and Aswan populations were grouped together in the first sub-cluster (similarity of 0.917), while the second one included El-Serow and Kafr El-Sheikh populations (similarity of 0.825). The second cluster comprised only El-Qanater population which is considered as the most distant one. These results could be useful in *Oreochromis niloticus* breeding programs.

Key words: *Oreochromis niloticus*, Biochemical markers, RAPD markers, Genetic relationships.

INTRODUCTION

Tilapia species were mainly produced from natural fisheries (lakes and rivers) as well as some governmental and private fish farms. However, four tilapia species are farmed in Egypt; Nile tilapia (*Oreochromis niloticus*), Blue tilapia (*Oreochromis aureus*), White tilapia (*Sarotherodon galilaeus*) and Green tilapia (*Tilapia zilli*). Tilapias represent the most important food fishes with potential for fish farming in tropical regions especially in Egypt. Commercial culture of tilapia is focusing on Nile tilapia and research has begun to overcome some of the main problems associated with farming this species. So, Nile tilapia comprises more than 90% of the farmed tilapias in Egypt because it is an ideal species for production systems since they might reach an acceptable size within the growing season of rice and most of the consumers prefer this species (Badawy, 1993).

Biochemical markers such as blood serum and muscle proteins as well as isozymes in blood and some organs of fish appear to be variable and can be used as markers in fish taxonomy (Kirpichinkov, 1973). Usually, esterase and some dehydrogenase isozymes (Adh, Ldh & Mdh) are commonly used as biochemical genetic markers for the identification of tilapia stocks (Cruz *et al*., 1982). Identification of biochemical (proteins and/or isozymes) markers for species taxonomy is based on subjecting tissue component samples of taxa under investigation for biochemical tests or assays. The validity of biochemical tests depends greatly upon the quality of samples. Several fish organ protein extracts were tested to detect ontogenic variations. Protein polymorphisms were used to calculate the similarity and genetic distance values as well as to construct the dendrogram among fish populations (Nxomani *et al*., 1994). The protein profiles for intra and inter-population identifications were successfully applied. Serum and muscle proteins are commonly used to assess the polymorphism

among fish species (Macaranas *et al*., 1996). However, Rashed *et al*. (2000) concluded that muscle protein was highly conserved which resulted in low intra variations in six Egyptian catfish locations.

Molecular genetic markers may shed light on the classification and defining the relationships among species and local populations. DNA markers analysis using Randomly Amplified Polymorphic DNA (RAPD) technique was used in the interpretation of the changes in germplasm of Nile tilapia in Egypt. To obtain maximal information about the potential interactions between farmed and wild populations, nucleotide sequence heterogeneity should be determined (Skaala *et al*., 1990). However, several molecular techniques were applied to detect DNA markers and to reflect the genetic background of fish populations. RAPD analysis had a value to assay polymorphisms within and among populations of three tilapia species. RAPD marker fingerprints under identical amplification and electrophoretic conditions were highly reproducible for any given primer – template combination (Bardakci and Skibinski, 1994). RAPD marker was used to obtain sequence differences between selected cichlid species. RAPD marker had a value in genetic characterization using bulked samples and so any marker revealed from these ways should be related to population not to any individual (Sultmann *et al*., 1995). Polymorphic DNA markers can provide fish researchers with new insight into the behavior, ecology and genetic structure of fish populations (Daguin and Borsa, 1999).

The aims of this investigation were to detect the degree of polymorphisms and genetic relationships within [using biochemical (water and alcohol soluble proteins and Est, Adh & Mdh isozymes) and molecular (10 RAPD primers) markers] and among [using the same 10 RAPD primers] five Egyptian Nile tilapia populations to provide a baseline data for future breeding programs.

MATERIALS AND METHODS

1. Materials.

Muscles and caudal fin samples of ten fish individuals representing five Egyptian Nile tilapia populations were collected from El-Qanater (Q), Kafr El-Sheikh (K), El-Serow (S), Aswan (A) and Naser Lake (N). However, El-Qanater and El-Serow populations represented two different governmental research farms, followed by Kafr El-Sheik population which represented a private production

farm, while Aswan and Naser Lake population individuals were collected from their natural habitat in the Nile River.

2. Methods.

2.1. Biochemical analyses.

2.1.1. SDS-PAGE analysis.

The ten collected muscle samples were used to detect both water and alcohol soluble protein polymorphisms within each of the five populations. Gel preparation, electrophoretic conditions, staining and destaining were performed according to Laemmli (1970).

2.1.2. Isozymes analysis.

The same collected samples were used to detect esterase (Est), alcohol dehydrogenase (Adh) and malate dehydrogenase (Mdh) isozyme polymorphisms within each of the five populations. The electrophoresis of these isozyme systems was performed according to McAndrew and Majumdar (1983).

2.2. RAPD analysis.

2.2.1. Genomic DNA isolation.

The ten collected caudal fin samples from each population were used to detect RAPD polymorphism within each of the five populations. Genomic DNA was isolated according to Sambrook *et al.* (1989). The genomic DNA of each ten samples from each population was mixed in a bulked sample according to Lukyanov *et al*. (1996) and used to detect RAPD polymorphism among the five tested populations.

2.2.2. RAPD conditions.

The amplification conditions and PCR mixture were set according to Williams *et al*. (1990). A set of ten decamer random primers as listed in Table (1) was used to detect the genetic relationships within and among the five tested populations. The amplified products (12.5µl loaded) were separated on 1.5% agarose gels and the amplified fragments were photographed under UV light using Polaroid Camera.

Table (1): Primer codes and sequences which used in the present study.

Primer code	Primer sequence 5'→3'	Primer code	Primer sequence 5'→3'
A06	GGTCCCTGAC	B08	GTCCACACGG
A07	GAAACGGGTG	C03	GGGGGTCTTT
A10	GTGATCGCAG	C05	GATGACCGCC
A19	CAAACGTCGG	C12	TGTCATCCCC
A20	GTTGCGATCC	C13	AAGCCTCGTC

2.3. Densitometric and statistical analysis.

Protein, Isozymes and RAPD profiles were analyzed and scanned for gel quantitation using GelDoc2000 instrument and quantity-one software package supplemented by the manufacturer (Bio-Rad). The banding profiles were scored in a binary manner where 1 indicates band presence while band absence was indicated by 0. The scored binary profiles were introduced into SPSS statistical software package to estimate both similarities and genetic distances for both within and among the five tested populations. A Dendrogram was constructed using pair-wise groups as a mathematical average of unweighed samples (UPGMA) based on the estimated data that was obtained from the analysis of the ten RAPD primer profiles.

RESULTS AND DISCUSSION

1. Genetic variations within the five tested populations.

1.1. Biochemical and RAPD polymorphisms.

The banding patterns within the five tested populations revealed wide variations of different bands for both biochemical (345 monomorphic and 293 polymorphic bands) and RAPD (173 monomorphic and 278 polymorphic bands) markers giving about 45.92% and 61.64% of polymorphism for biochemical and RAPD markers, respectively, with an average of 53.78% which indicated a moderate genetic variation within each of these populations as shown in Table (2). However, there was a clear coincidence among polymorphism percentages of biochemical and RAPD markers and their average, where El-Qanater population showed the highest value, followed by El-Serow, Kafr El-Sheikh and Naser Lake populations, while Aswan population revealed the lowest value.

Table (2): Polymorphism percentages within the five tested populations based on biochemical and RAPD markers.

Population	Biochemical markers			RAPD markers			Polymorphism percentage average
	Total bands	Polymorphic bands	Polymorphism percentage	Total bands	Polymorphic bands	Polymorphism percentage	
El-Qanater	141	80	56.74%	103	76	73.79%	65.27%
Kafr El-Sheikh	126	57	45.24%	87	55	63.22%	54.23%
El-Serow	131	66	50.38%	91	58	63.74%	57.06%
Aswan	121	42	34.71%	87	42	48.28%	41.50%
Naser Lake	119	48	40.34%	83	47	56.63%	48.49%
Total	638	293	45.92%	451	278	61.64%	53.78%

1.2. Similarity matrix within the five tested populations.

The ranges and averages of similarity values within each of the five tested populations based on biochemical and RAPD markers are listed in Table (3). Based on biochemical markers, all tested populations showed high similarity averages within each one, which indicated high homogeneity within each population. However, the highest similarity average was found within Naser Lake population (0.994), while the lowest one was recorded within El-Serow population (0.968). Concerning RAPD markers, the highest similarity average was observed within Aswan population (0.840), followed by El-Serow (0.829), Naser Lake (0.807) and Kafr El-Sheikh (0.785) populations, while the lowest average was detected within El-Qanater population (0.745). Moreover, the mean of the genetic similarity averages within each tested population based on biochemical and RAPD markers showed that Aswan population had the highest value (0.916), followed by Naser Lake (0.901), El-Serow (0.899) and Kafr El-Sheikh (0.886) populations, while the lowest value was found within El-Qanater population (0.863).

Mean of the similarity averages within each tested population based on biochemical and RAPD markers reflected the genetic structure of each population. The highly detected homogeneity value within Aswan population could be attributed to the inbreeding occurred in this area of Nile River. While EL-Qanater population showed the lowest value which could be resulted from the outbreeding occurred between this population and other populations presented in

the nearest Nile River channels (such as Darawa and El-Menofy). In this respect, Hulata *et al.* (1986) reported that genetic bottle necks and a high level of inbreeding may significantly limit the genetic variation and marginal response to genetic improvement, especially when a small founder population was used. Moreover, mean of the similarity averages within all tested populations based on biochemical and RAPD markers and their average were 0.984, 0.801 and 0.893, respectively, which indicated that RAPD marker was more prominent for illustrating the genetic structure and detecting the degree of homogeneity and inbreeding within each tested population.

Table (3): Similarity ranges and averages within each of the five tested populations based on biochemical and RAPD markers.

Population	Biochemical markers		RAPD markers		Average mean
	Range	Average	Range	Average	
El-Qanater	0.900-1.00	0.980	0.636-0.899	0.745	0.863
Kafr El-Sheikh	0.970-1.00	0.987	0.677-0.900	0.785	0.886
El-Serow	0.844-1.00	0.968	0.704-0.900	0.829	0.899
Aswan	0.970-1.00	0.991	0.769-0.929	0.840	0.916
Naser Lake	0.970-1.00	0.994	0.757-0.875	0.807	0.901
Average mean		0.984		0.801	0.893

Rashed *et al.* (1998) concluded that isozymes, especially Est, had a power for detecting genetic relationships within and among some Egyptian Nile tilapia populations. Moreover, Rashed *et al.* (2000) found that intra-location variations of muscle proteins in six Egyptian catfish populations were low, which is considered as species-conserved protein. In addition, Elghobashy (2002) found that intra-specific variations of muscle protein in four Egyptian Nile tilapia populations were low for both Abbassa and Manzala populations, while they were high for both Maryout and Aswan populations. Recently, Rashed *et al.* (2007) found that genetic similarity matrix based on muscle protein profiles was high within each four Egyptian *Hemichromis bimaculatus* populations.

2. Genetic variations among the five tested populations.

2.1. RAPD polymorphism.

Out of 84 amplified fragments, 56 were polymorphic fragments (66.67% of polymorphism) among the bulked individual DNAs of the five tested populations by the ten used primers as shown in Figure (1)

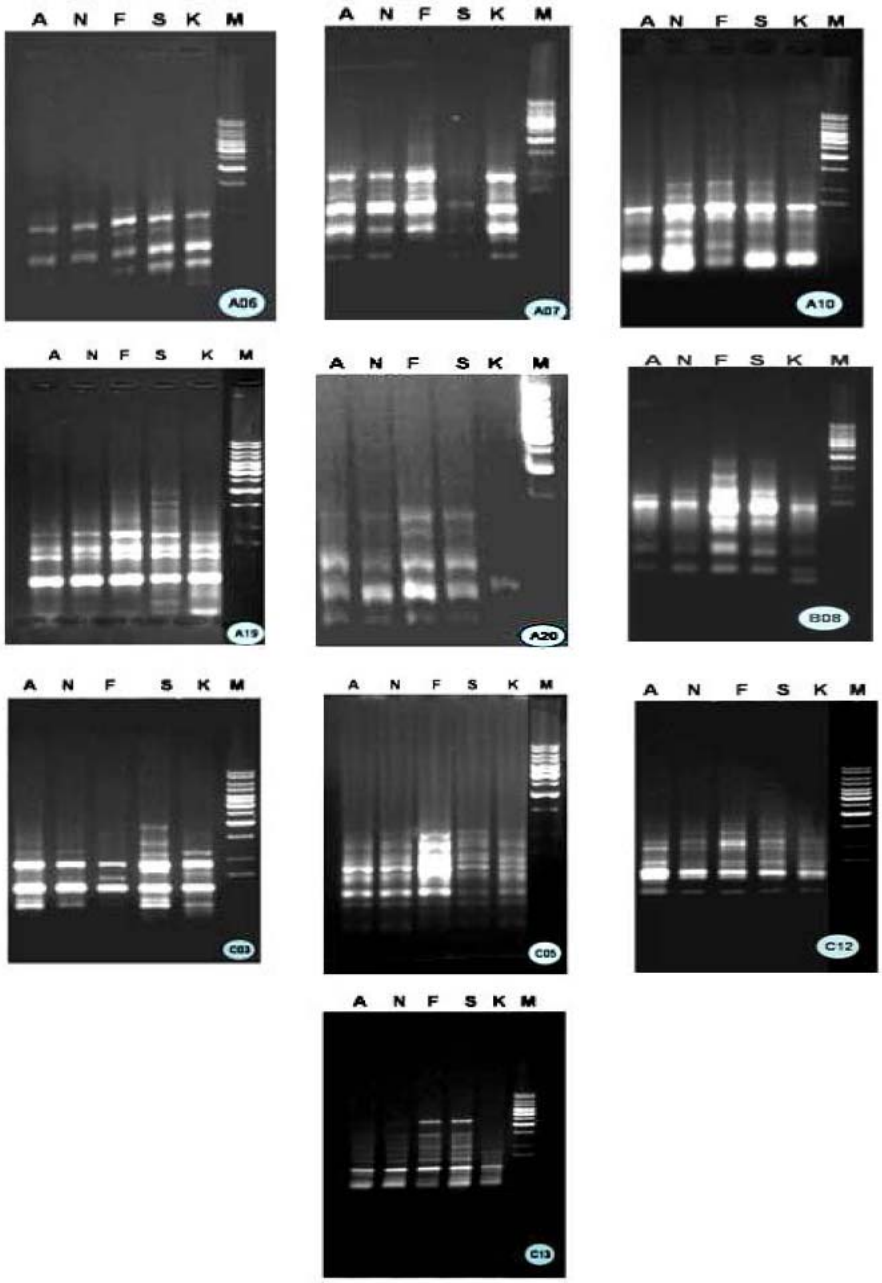

Figure (1): RAPD-PCR fragments of A06, A07, A10, A19, A20, B08, C03, C05, C12 and C13 primers for the ten used bulked individual DNAs of the five tested populations.

and Table (4). The ten used primers showed different levels of polymorphism percentages. However, the highest polymorphism percentage was recorded for A06 (100.0%) primer, followed by B08 (90.91%), A19 (88.89%), C13 (87.50%) and A20 (80.00%) primers. Moreover, a moderate polymorphism was recorded for A07 (62.50%), A10 (42.86%) and C12 (40.00%) primers. On the other hand, the lowest polymorphism percentage was recorded for C05 (16.67%) primer.

Table (4): Number of amplified fragments and polymorphism percentages among the five tested populations using 10 primers.

Primer	Number of amplified fragments / population					Total number of fragments	Polymorphic fragments	Polymorphism percentage
	Q	K	S	A	N			
A06	1	3	3	1	2	5	5	100.00%
A07	6	5	4	5	5	8	5	62.50%
A10	5	7	6	5	7	7	3	42.86%
A19	4	4	5	3	3	9	8	88.89%
A20	1	5	5	4	4	5	4	80.00%
B08	3	10	8	3	4	11	10	90.91%
C03	2	3	6	3	3	9	8	88.89%
C05	10	12	10	11	11	12	2	16.67%
C12	9	9	9	7	9	10	4	40.00%
C13	2	6	6	3	3	8	7	87.50%
Total	43	64	62	45	51	84	56	66.67%

2.2. Similarity matrix and genetic relationships among the five tested populations.

The similarity matrix among the five tested populations based on all primer profiles showed different values with an average of 0.741 which indicated a moderate homogeneity among these populations as shown in Table (5). However, the highest similarity value was observed between Aswan and Naser Lake (0.917) populations, followed by that between Kafr El-Sheikh population and both El-Serow (0.825) and Naser Lake (0.783) populations, while the lowest one was between El-Qanater and Kafr El-Sheikh (0.617) populations. Moreover, the other values were moderate and ranged from 0.743 between El-Serow and Naser Lake populations to 0.667 between El-Qanater and El-Serow populations.

Table (5): Similarity matrix among the five tested populations based on overall RAPD primers.

Population	El-Qanater	Kafr El-sheikh	El-Serow	Aswan
Kafr El-sheikh	0.617	1.000		
El-Serow	0.667	0.825	1.000	
Aswan	0.727	0.734	0.692	1.000
Naser lake	0.702	0.783	0.743	0.917

The dendrogram based on all RAPD primer profiles among the five tested populations was classified into two main clusters, where the first one was divided into two sub-clusters as shown in Figure (2).Within the first cluster, Naser Lake and Aswan populations were grouped together in the first sub-cluster, while the second one included El-Serow and Kafr El-Sheikh populations. The second cluster comprised only El-Qanater population which is considered the most distant population.

The results of similarity matrix and constructed dendrogram among the five tested populations based on all RAPD markers showed that Kafr El-Sheikh and El-Serow populations (which represented two different farms) initially got their tilapia fish stocks from Naser Lake (Which is considered as the main source of tilapia in Egypt), while EL-Qanater population (which also represents a farm) got its tilapia fish stock from a different source along the Nile River in Egypt.

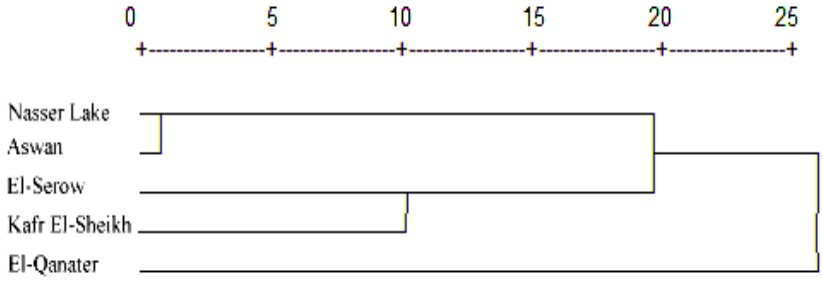

Figure (2): Genetic distances among the five tested populations based on all RAPD primer profiles.

Falk *et al.* (1996) noticed the limitation of taxonomic value for some Tilapiine species such as *O. niloticus*, *S. gallilaeus* and *T. Zilli* using water soluble muscle proteins. They concluded that taxonomic value of parvalbumins, which are abundant in the white muscles,

based on their molecular weight differences is limited among closely related tilapiine species. Moreover, Palti *et al.* (1997) reported that DNA fingerprints of bulked DNA samples can be useful in breeding programs for assessing genetic relationships among closely related populations due to the high level of genetic differentiation detected by these markers. In addition, Rashed *et al.* (2007) detected genetic relationships among four *Hemichromis bimaculatus* populations namly; El-Qanater, Wadi El-Rian and Manzala Lake based on muscle protein polymorphism. The constructed Dendrogram indicated that El-Qanater population was distantly related from the others. Finally, Rashed *et al.* (2008) found a relatively high level of genetic relationships among three Egyptian Nile tilapia populations namly; Naser Lake, Giza and El-Qanater using RAPD markers, which is required for these populations to be more adaptive with the environmental changes.

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علاقات القرابة الوراثية داخل وبين خمسة عشائر من البلطي النيلي في مصر بإستخدام العلامات البيوكيماوية والجزيئية

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إستخدمت خمس عشائر من البلطي النيلي (القناطر وكفر الشيخ والسرو وأسوان وبحيرة ناصر) لتحديد مدى قابلية بعض العلامات البيوكيماوية (البروتينات الذائبة في الماء والكحول وأنزيمات الاستيريز والكحول ديهيدروجينيز) والجزيئية على مستوى الـ DNA (بإستخدام البادئات A06, A07, A10, A19, A20, B08, C03, C05, C12, C13) لتحديد الإختلافات وعلاقات القرابة الوراثية داخل هذه العشائر. تم اخذ عشرة عينات من كل عشيرة لتلك التحليلات البيوكيماوية والجزيئية. أظهرت التحليلات البيوكيماوية 45,92% والجزيئية 61,64% من نسب تباين الحزم الوراثية بمتوسط حوالي 53,78% والذي يؤكد وجود إختلاف معتدل داخل كل عشيرة من تلك العشائر. وقد ظهر ايضاً أن متوسط مدى التشابه الوراثي داخل كل العشائر المدروسة بإستخدام تلك التحليلات البيوكيماوية كان 0,984 وفي الجزيئية كان 0,801 بمتوسط مقداره 0,893 والذي يؤكد أن التحليلات الجزيئية كانت أفضل لتوضيح التركيب الوراثي ولتحديد درجة التماثل والتربية الداخلية داخل كل عشيرة من العشائر المدروسة. ومن ناحية أخرى فقد تم إستخدام نفس العشرة بادئات لتحديد الإختلافات وعلاقات القرابة خلال تلك العشائر الخمس بالتفاعل مع المادة الوراثية المخلوطة للعشرة عينات المأخوذة من كل عشيرة على حدى. وقد أظهر هذا التحليل مستويات مختلفة من نسب تباين الحزم الوراثية بمتوسط حوالي 66,67% والذي يتراوح من 100% بإستخدام البادىء A06 إلى 16,67% بإستخدام البادىء C05. وقد أكدت أيضاً قيم التماثل الوراثي وشجرة القرابة خلال تلك الخمسة عشائر بإستخدام ذلك التحليل أن عشيرتي بحيرة ناصر واسوان كانا أكثر تقارباً وراثياً (0,917 نسبة تماثل) يليهم عشيرتي السرو وكفر الشيخ (0,825 نسبة تماثل) في حين كانت عشيرة القناطر الأكثر تباعداً وراثياً. قد تعتبر هذه النتائج مفيدة في برامج التربية للبلطي النيلي في مصر.