

Monitoring of Genetic Polymorphism in Some Tilapia Species Based on Fin Tissues Isozyme Distributions

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Abstract: Monitoring of genetic polymorphism in *Tilapia zillii* (Z), *Sarotherodon galilaeus* (A) and *Oreochromis niloticus* (N) were carried out in five different fin tissues. Three isozyme systems (Esterase, Malate dehydrogenase and Malic enzyme) were assayed. The couple means of observed heterozygosity and percentage of detected polymorphic alleles were 0.42 and 42.86%; 0.47 and 47.62% and 0.57 and 57.14% in Z, A and N, respectively. The lowest similarity value (0.76) among the studied fish species was observed between Z and N. The current study offered a useful tool for studying fish genetic structure based on isozyme polymorphism in external body organs (Fins) which can be extracted by biopsy.

Key words: Tilapia · Isozyme · Genetics · Characterization · Heterozygosity

INTRODUCTION

The genetic markers should be conducted to provide the information needed for a sound management of fish (such as Tilapia fish) farming and wild fish stocks. This way will be useful, especially in fish breeding programs which use genetic markers as a marker assisted selection to improve the fish performance and/or economic traits [1]. Several techniques have been used, with different degree of success, to monitor and tracing minor differences among fish populations, genera, species and individuals [2]. Among these techniques, however, biochemical and molecular studies are advantageous [3, 4]. Allozyme electrophoresis have long been the standard genetic tools in phylogenetic and taxonomic research, since allozyme alleles are co-dominant markers. Most of fish organ extracts were used as isozyme sources for fish characterization in many studies (Fins were not included) such as [2]. They found that, ontogenic variations based on isozymes have a power for detecting genetic characterization for *O.niloticus* populations. Isozyme variability has also been shown to be a good indicator of overall heterozygosity and can be used to assess the degree of inbreeding in a particular population [5].

The aim of this study was to monitor the genetic polymorphism in some Tilapia species (*Tilapia zillii*,

Sarotherodon galilaeus and *Oreochromis niloticus*) by fin tissues isozyme (Est, Mdh and ME) distributions. This study hope to offer a useful scope for studying fish genetic structure based on gene expressions in external body organs (Fins) which can be extracted by biopsy.

MATERIAL AND METHODS

Sample Collection: Three Tilapia species (according to Trewavas [6], *Tilapia zillii* (Z), *Sarotherodon galilaeus* (A) and *Oreochromis niloticus* (N) were collected during year 2007 from Nasser Lake near Aswan (A, 23.5°N 32.8°E). A total of 20 fish samples (10 males and 10 females) from each examined species was used for studying the genetic structure. All fish fins (five fins; caudal (c), dorsal (d), pelvic (pl), pectoral (p) and anal (a)) from all individuals were tested.

Isozyme Extraction and Electrophoresis: Isozyme extractions and purifications were carried out as discribed by Rashed *et al.* [5] with some modifications (0.3 g of each powdered fish fin was extracted in 350 µl of 0.85% NaCl solution).

Esterase with five synthetic substrates (α -naphthylpropionate, α -naphthylacetate, β -naphthylacetate, α -naphthylvalerate and α -naphthylbuterate), Malate dehydrogenase (Mdh) and

Malic enzyme (ME) were employed to discriminate isozyme variations in the three applied Tilapia fish. Electrophoretic conditions, gel preparation, staining and distaining were conducted according to Rashed *et al.* [5], Tanksley and Orton[7] and Tanksley and Rick [8].

Data Analysis: Gels were scanned using Bio-Rad Gel-Doc 2000. The data were analyzed with *POPGENE* program version 1.32 [9]. The estimated parameters were: Polymorphic alleles, allele frequency, proportion of polymorphic loci, heterozygosity, genetic distance, genetic similarity and dendrogram construction.

RESULTS

Fin Isozyme Distributions: A total of three isozyme systems (EST, Mdh and ME) was examined. Number of detected bands and average of allele frequencies were calculated in five fish fins (c, d, pl, p and a) for each studied fish species (Table 1). All the examined fins shared some isozyme bands while some bands were absent in certain fins. The averages of band frequency based on fins isozyme polymorphism were 0.74, 0.76, 0.79, 0.80 and 0.81 in (c), (d), (p), (Pl) and (a) fins, respectively (Table 1).

No, differences were noted between males and females within each studied fish species across all estimated fish fins.

Esterase Enzyme (Est.): The distribution of some esterase bands were varied in different fin types, some of these bands were species specific.

In all studied fish fins, the esterase reactions with α -naphthylpropionate (α -n.p.), α -naphthylacetate (α -n.a.), β -naphthylacetate (β -n.a.), α -naphthylvalerate (α -n.v.) and α -naphthylbuterate (α -n.b) substrates produced 4, 3, 3, 3 and 5 anodally migrated bands, respectively (across all studied Tilapia species). The highest number of detected esterase alleles (6) and lowest average of allele frequency (0.71) were detected in (c) fin using all esterase substrates (Table 1). The results indicated that in these Tilapia species, there is multiplicity of genetic specification with respect to esterase isozymes.

Malic Enzyme (ME): Four anodally migrated ME bands were recognized in all fins across all studied Tilapia species. The average of band frequencies were 0.92, 0.58, 0.91, 1 and 1 in (c), (d), (p), (pl) and (a) fins, respectively (Table 1). The Pattern of ME tissue specific gene expressions was quite variable except in (d) case.

Table 1: The average of band frequency and number of detected bands according to its relative mobility based on fin isozyme polymorphism

Isozyme System	c		d		p		pl		a	
	BNR	ABF	BNR	ABF	BNR	ABF	BNR	ABF	BNR	ABF
Est. α -n.a	5-6	0.62	4-5	0.93	4-5	0.73	5-5	0.74	4-5	0.86
Est. α -n.p.	3-4	0.90	3-4	0.91	3-4	0.91	3-4	0.91	3-3	0.74
Est. α -n.v.	2-3	0.88	2-2	0.66	2-2	0.66	2-2	0.66	2-2	0.66
Est. α -n.b.	2-3	0.55	1-3	0.66	1-3	0.66	1-3	0.66	1-3	0.66
Est. β -n.a.	4-5	0.60	4-4	1	4-4	1	4-4	1	4-4	0.91
Average		0.71		0.83		0.79		0.79		0.76
ME	3-4	0.92	1-2	0.58	3-4	0.91	4-4	1	3-3	1
Mdh	2-3	0.77	3-6	0.61	2-4	0.66	2-4	0.66	2-4	0.83
Average		0.74		0.76		0.79		0.80		0.81
SE		0.06		0.06		0.05		0.06		0.04

BNR = Range number of band detected, ABF = average of band frequency and SE = standard error

Table 2: Actual number of alleles (na), effective number of alleles (ne), Shannon's information index (I), Observed heterozygosity (Obs. H), Expected heterozygosity (Exp. H) and percentage of polymorphic alleles (%PL) for each studied Tilapia species (Mean + SE)

	na	ne	I	Obs. H	Exp. H	%PL
<i>T.zillii</i>	1.42±0.02	1.42±0.02	0.29±0.01	0.42±0.02	0.22±0.01	42.86
<i>S.galilæus</i>	1.47±0.02	1.47±0.02	0.33±0.01	0.47±0.02	0.25±0.01	47.62
<i>O.niloticus</i>	1.57±0.02	1.57±0.02	0.39±0.01	0.57±0.02	0.30±0.01	57.14

Table 3: Nei's genetic similarity (above diagonal) and genetic distance (below diagonal)

Case	Z	A	N
Z		0.77	0.76
A	0.25		0.83
N	0.26	0.18	



Fig. 1: Dendrogram represent the inferred phylogenetic relationships among studied Tilapia species Z=*T. zillii*, A=*S. galilaeus* and N=*O. niloticus*. The lengths among sites were 13.32, 3.58, 9.73 and 9.7 between (2,Z), (2, 1), (1, A) and (1, N), respectively.

Malate Dehydrogenase (Mdh): Four anodally Mdh migrated bands were recognized in all fins across all studied Tilapia species. The average of band frequency were 0.77, 0.61, 0.66, 0.66 and 0.83 in (c), (d), (p), (pl) and (a) fins, respectively (Table 1). Two isoforms (mMdh and sMdh) are detected in all fish fins across all studied fish species. mMdh isozymes have lower mobility than sMdh. The mMdh was detected in all studied fins (across all examined fish species), while the sMdh was expressed in most fin types. The Pattern of Mdh tissue specific gene expressions was variable in all studied fins.

Genetic Polymorphism Within Each Studied Tilapia Species: The genetic structure of each studied fish species was estimated based on genetic polymorphism in gene expressions in caudal fins (c). The (c) fin was chosen here because it had the lowest average of allele frequencies (0.74). The heterozygosity values for all loci were averaged and presented in Table (2). The mean of observed and expected heterozygosities were calculated in each studied Tilapia species based on the 21 examined loci. They were (0.42 and 0.22), (0.47 and 0.25) and (0.57 and 0.30) in (Z), (A) and (N) species, respectively.

The (c) fin allozyme frequencies were ranged from 0.5 to 1. The mean of actual number of alleles (na), effective number of alleles (ne), Shannon's information index (I) and percentage of polymorphic alleles (%PL) for each studied Tilapia species were also estimated. The percentage of detected polymorphic alleles were 42.86, 47.62 and 57.14% in (Z), (A) and (N) populations, respectively. Among studied Tilapia species, the highest I (0.39), ne (1.57) and na (1.57) values were detected in (N) while the lowest values of these parameters were detected in (Z) as presented in (Table 2).

Genetic Polymorphism for All Loci among Studied Tilapia Species: The lowest similarity value (Table 3) was observed between *T.zillii* and *O.niloticus* (0.76) while the highest similarity value was observed between *S. galilaeus* and *O.niloticus* (0.83). The results generated from the isozyme polymorphism were pooled to draw the

genetic relationships among the three examined Tilapia species. The constructed dendrogram (Figure1) reflects the genetic distance among the applied fish species which agree with the similarity values studied among studied fish species.

DISCUSSION

Isozyme polymorphism are widely used as biochemical genetic characterization in most fish species, natural populations and cultured fish strains such as in Tilapia [1, 5] and *H. bimaculatus* [10]. From the previous studies, Esterase, ME and Mdh isozyme systems were recommended in detection of fish characterization. So, ME, Mdh and Est were tested in the present study in order to trace reliable comparisons among and within the three studied Tilapia species. In the current study the most reliable patterns were obtained in the case of esterase isozymes in (c) fin. The lowest similarity value was observed between *T.zillii* and *O.niloticus* while the highest similarity value was observed between *S.galilaeus* and *O.niloticus*. In addition, the population analysis was carried out based on the caudal fin (c) isozyme polymorphism, because the (c) fin isozyme polymorphism had lowest average of allele frequencies (0.74).

Some esterase bands were found to be active in all investigated fin tissues. Such wide distribution suggests that these esterase bands could be housekeeping enzymes [11] which serve a general metabolic function characteristic of all cell types. The esterases are a complex class of enzymes, where electrophoretic expression depends on the choice of substrate. The substrate tissue-specific differences in esterase spectra have been recorded where the distribution of some esterase bands was varied in different fin tissues and some of these bands were fin tissue-specific. The same conclusion was reported by Rashed *et al.* [5] in Tilapia (*O. niloticus*, *O. aureus* and *T. zillii*) muscles isozymes and also noted in *H. bimaculatus* muscles [10]. In all of the previous studies, fish muscle isozyme polymorphism was

recommended for fish characterization. In this way, fish should be killed to obtain the muscle samples for isozyme extractions. Rashed *et al.* [2] focused on tissue isozyme distributions among ten fish body organs (Fins were not included). The most reliable patterns were obtained in the case of esterase isozymes in muscle. The distinct profiles were always included in the case of esterase with propionate reaction and mostly in regard to butyrate reaction. The valerate derivative displayed often the fewer patterns. This conclusion was confirmed by Rashed *et al.* [5]. In the present study all of tested fin esterase substrates pattern gave sharp, distinct banding and intense resolutions. Post translation modification might regulates the expression of this locus. Therefore, some products (bands) of this locus are commonly distributed (all fish fins) while others are restricted in their fin tissue distributions.

The Pattern of tissue specific gene expressions of both ME (four subunits) and Mdh (two subunits) isozymes were variable. The variability levels in Mdh were higher than in ME. This note was confirmed by the results of allele frequency, where the average of allele frequencies based on ME was higher than Mdh (except in d fin). In eukaryotes, two Mdh isoforms were detected (m-Mdh and sMdh). In addition, genomes of many fish encode two sMdh isoforms. mMdh isozymes have lower mobility than sMdh ones [12]. In the present study, the two isoforms (mMdh and sMdh) were detected in all fish species. With regard to fin tissue specific enzyme, the mMdh was detected in all studied fins across all studied fish species while the sMdh was expressed in certain fin types. Our results showed that, possibly two Mdh loci encoding sMdh and the Pattern of fin tissue specific gene expression of sMdh isozymes was variable.

the current results suggested that, the genetic variability within (N) was higher than both other two studied Tilapia species (Z and A) because the (na), (ne), (I) and (%PL) values estimated in (N) were higher than the other two studied Tilapia species (A and Z).

In conclusion, there is multiplicity of genetic specification with respect to esterase isozymes in tilapia species. The present study offered a useful scope for detecting species specific biochemical genetic markers using external body organs (Fins) extracts without killing the fish. This way will be useful value, especially in fish breeding programs which use genetic markers as a marker assisted selection to improve the fish performance.

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REFERENCES

1. PNGS (Producing pure lines of the Egyptian Nile tilapia using molecular genetic techniques and selection). 2007. Program of National Strategy for Biotechnology and Genetic Engineering, Science and Technology Center, Academy of Scientific Research and Technology, Ministry of Scientific Research, Egypt.
2. Rashed, M.A., A.A. EL-Gamal, T.M. Tantawy and Y.M. Saad, 1998. Detection of *Oreochromis niloticus* lines by isozyme organs distribution and RAPD-PCR DNA markers. 3rd Arab Conference Modern Biotech. And Area of Application in the Arab world, 14-17 Dec., Cairo, Egypt. pp: 127-146.
3. Macaranas, J.M., L.Q. Agustin and A.E. Eknath, 1996. Multiple haemoglobine in three Tilapiine species of the genus *Oreochromis* and in eight strains of *O. niloticus* L. Aquaculture Res., 27: 597-601.
4. Rashed, M.A., Y.M. Saad, M.M. Ibrahim and A. El-seoudy, 2008. Genetic Structure of Natural Egyptian *Oreochromis niloticus* Evaluated Using Dominant DNA Markers. Global Vet., 2: 87-91.
5. Rashed, M.A., Y.M. Saad, S.I. EL-Deeb, A.A. EL-Gamal and M.M. Saiid, 2002. Characterization of some Egyptian Tilapia populations based on protein subunits and specified substrates of muscle esterase. 1st Scientific Conference of the Egyptian Aquaculture, El-Arish, North Sinai. 13-15 Dec. pp: 364-378.
6. Trewavas, E., 1983. Tilapiine fishes of the genera *Sarotherodon*, *Oreochromis* and *Danakilia*. British Museum (Natural History). London, Uk. pp: 583.
7. Tanksley, D. and C. Rick, 1980. Genetics of esterases in species of Lycoporicon. Theoretical Applied Genetic, 56: 209-219.
8. Tanksley, S. and T. Orton, 1983. Isozymes in plant genetic and breeding. Part (B). Elsevier Science Publishers B.V. Amsterdam.

9. Yeh, F. and T. Boyle, 1997. Population genetic analysis of co-dominant and dominant markers and quantitative traits. *Belgian Journal of Botany*: 129: 157. A joint Project Development by Francis C. Yeh and Rong-cai Yang, University of Alberta and Tim Boyle, Centre for International Forestry Research.
10. Rashed, M.A., E.A. Badaway, Y.M.S. and A.B. Abd EL-Razek, 2007. Genetic signature of some Populations of the Egyptian fish *Hemichromis bimaculatus* based on the muscle protein polymorphism. *Egyptian J. Aquatic Biol. Fisheries*, 11(1): 83-100.
11. Holmes, R.S. and G.S. Whitte, 1970. Developmental genetics of the esterase isozymes of fundulus heteroclitus. *Biochemical Genetic*, 4: 471-480.
12. Bailey, G.S., A.C. Wilson, J.E. Halver and C.L. Johnson, 1970. Multiple form of supernatant malate dehydrogenase in Salmonid fishes: Biochemical, immunological and genetic studies. *J. Biological Chem.*, 245: 5927-5940.