

COMPARATIVE ELECTROPHORETICAL STUDIES OF THE SARCOPLASMIC PROTEINS OF TWO *OREOCHROMIS* SPECIES FROM DIFFERENT LOCALITIES

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Abstract

Sarcoplasmic proteinograms of both sexes of *Oreochromis niloticus* and *O. aureus* from Manzalla lake, River Nile (Benha area) and private fish farm (Sharkia province) were fractionated using 7.5% polyacrylamide gel electrophoresis. Polymorphism, relative mobility and similarity coefficient (SC) between mobilities of fractions were studied.

Three fractions (#9, 10 and 13) absolutely appeared in all individuals. Thirteen fractions with different degrees of polymorphism were detected for both species.

Different relative mobility values were recorded and significant sex variations were shown in the fraction #1 of *O. niloticus* from the river Nile and Fraction #4 of *O. aureus* from Manzalla lake.

Highest similarity coefficients were calculated in both sexes of *O. niloticus* from Manzalla lake and that from the river Nile. SC values were lower in specimens from fish farms than those from either Manzalla lake or river Nile. This indicated that, the relative genetic purity (from the biochemical point of view) was higher for *O. niloticus* from the river Nile, followed by that from Manzalla, while, that from the private fish farm had the lowest one.

When comparing the two species of the same locality, very highly SC were detected in both sexes from Manzalla lake, while, less SC were observed in the private fish farm.

INTRODUCTION

Wohlfarth and Hulata (1981) reported that the prolific spawning of tilapia causes overcrowding in the rearing ponds, and the production of fingerlings of

all-male tilapia hybrids is the best solution to this problem. This could be obtained by hand sexing, sex reversal using hormones or intercrossing between two different species of tilapia (Rothbard & Pruginin 1975 and Badawy 1993). *Oreochromis niloticus* and *O.aureus* are the most dominant among tilapia species in many Egyptian water bodies (Payne and Collinson 1983). The genetic purity of the parents of female *O.niloticus* and male *O.aureus* plays an important role in the male ratio of their produced hybrid progenies (Badawy 1993).

Estimating genetic variability in a fish population depends mainly on three methods: biometric, chromosomal and biochemical. Studying muscle protein (Myogen) of fish using electrophoretic technique is one of the biochemical methods used to differentiate between fish species (Herzberg and Pasteur 1975 and El-Saied 1993).

This work aimed to determine the changes in the number and mobility of the biochemical markers of the sarcoplasmic proteins of *O.niloticus* and *O.aureus* from three different localities, using polyacrylamide gel electrophoresis, to be a guide for those interested in producing all-male tilapia hybrids.

MATERIALS AND METHODS

Twenty-one alive specimens of *Oreochromis niloticus* were collected from the river Nile (Benha area) where *O.aureus* not or rarely present, 18 from Manzalla lake (brackish area) and 12 from a private fish farm (near San El-Hagar, Sharkia province) which is supplied with water from Ramsis drain. Also, 17 and 20 alive *O.aureus* from the last two areas were gathered, respectively.

One gramme of flesh (left side below the dorsal fin) from each specimen was homogenized with 3 ml of cold distilled water and centrifuged at 5000 rpm for 15 minutes. The water-soluble protein extracts were fractionated using 7.5% polyacrylamide gel electrophoresis (Herzberg and Pasteur 1975). Gels were stained by Amido black 10B and destained by 7% acetic acid. The sarcoplasmic protein fractions were scanned by photoelectric densitometer model Gelman DCD-16.

Polymorphism of sarcoplasmic protein fraction was designed as the percentage frequency of appearance of each fraction in the electrophoretic patterns of each species (Payne *et al.* 1971).

The similarity between mobilities of fractions of the electrophoretic pattern

of members of the same species, or between those of the two species from different localities was assessed by simple matching coefficient of similarity (SC) according to the formula of Ferguson (1980):

$$\text{Similarity coefficient (SC)} = \frac{\text{Number of bands of common mobility}}{\text{Maximum number of bands in an individual}}$$

The lowest similarity coefficients are found between the most distant taxonomically pairs of species, while, the highest one are found between the closest taxonomically pairs of species (Ferguson 1980).

Statistical analysis of data was done according to Snedecor and Cochran (1976).

RESULTS

Fractions 9,10 and 13 absolutely (100%) appeared in all individuals of *Oreochromis niloticus* and *O.aureus*. *O.niloticus* from the three localities {Lake Manzalla (M), Private fish farm (F) and Benha area of the river Nile (N)}, and *O.aureus* from the two localities [Lake Manzalla (M) and Private fish farm (F)] showed 13 sarcoplasmic protein fractions with different degrees of polymorphism (Table 1).

For *O.niloticus*, different degrees of polymorphism were detected in both sexes from the three localities. Polymorphism was shown in 4 fractions (#2, 5, 8 & 12), and 5 fractions (#2, 3, 5, 7, & 8) in both males and females from Manzalla lake, respectively. From private fish farm, fractions 7, 8 & 12 showed polymorphism in both males and females in addition to fraction #3 (85.7%) in males only. From river Nile, females *O.niloticus* showed polymorphism only in fraction #3 (50%), and fractions 2&5 disappeared completely from all samples. However, their males were polymorphic in 7 fractions (#2, 3, 5, 7, 8, 11 & 12), and constantly, (90-99.9%) appeared in fractions 1&6 (Table 1).

The electrophoretic pattern of muscle proteinograms of both sexes of *O.aureus* from Manzalla lake showed polymorphism in fractions 2, 3, 4, 5, 8 & 12, and in fraction 11 (87.5%) in case of males only. On the other hand, males from the private fish farm showed 3 polymorphic fractions (#1, 3&7); one fraction (#5) constant and 2 fractions (#8 & 12) disappeared completely. The fractions 1, 3, 5, 7, 8 & 12 of their females were polymorphic (Table 1).

From the relative mobility point of view (Table 2), different mobility values

Table 1. Comparative identification map, showing the frequency of appearance of individual sarcoplasmic protein fractions for different experimental groups of *Oreochromis* species.

Group	Sex	Number of samples	Number of fractions														
			1	2	3	4	5	6	7	8	9	10	11	12	13		
Manzalla Lake : O. niloticus	M.	12	a	P(66.7)	a	a	P(83.3)	a	P(83.3)	a	P(75.0)	a	a	a	a	P(58.3)	a
	F.	6	a	P(66.7)	P(83.3)	a	a	P(66.7)	a	P(33.3)	a	a	a	a	a	a	a
O. aureus	M.	8	a	P(75.0)	P(37.5)	P(87.5)	a	P(50.0)	a	P(87.5)	a	a	a	a	a	P(87.5)	a
	F.	9	a	P(77.8)	P(77.8)	P(88.9)	a	P(55.6)	a	P(66.7)	a	a	a	a	a	P(62.5)	P(44.4)
Private fish Farm (Skarkia): O. niloticus	M.	7	a	a	P(85.7)	a	a	a	a	P(57.1)	P(28.6)	a	a	a	a	P(42.9)	a
	F.	5	a	a	a	a	a	a	a	P(60.0)	P(20.0)	a	a	a	a	P(60.0)	a
O. aureus	M.	11	P(81.8)	a	P(18.2)	a	a	c	a	P(9.1)	d	a	a	a	a	a	d
	F.	9	P(88.9)	a	P(55.6)	a	a	P(88.9)	a	P(55.6)	P(55.6)	a	a	a	a	a	p(44.4)
River Nile (Benha): O. niloticus	M.	15	c	P(80.0)	P(80.0)	a	a	P(20.0)	c	P(73.3)	P(73.3)	a	a	a	a	P(66.7)	a
	F.	6	a	d	P(50.0)	a	a	d	a	a	a	a	a	a	a	a	a

a : Absolute : found in 100% of individuals.

c : Constant : found in 90-99.9% of individuals.

P : Polymorphic : found in a part of individuals (less than 90%).

d : Disappeared : Completely disappeared in all individuals.

M. : Male individuals

F. : Female individuals

were recorded. Comparison of sexes in the two species and in the same locality showed that sarcoplasmic protein fraction # 1 of *O.niloticus* from the river Nile, and fraction #4 of *O.aureus* from Manzalla lake were significant with $P < 0.05$ and < 0.01 respectively.

Comparing the relative mobility of sarcoplasmic proteinograms of *O.niloticus* (Table 3) between the three localities showed that the similarity coefficients (SC) were higher in specimens from Manzalla lake than those from the river Nile (0.92 for males and 0.85) for females). SC values were lower in specimens from fish farm than those from either Manzalla lake or river Nile. On the contrary, *O.aureus* from Manzalla lake, when compared with that from the fish farm recorded high SC (0.85 for males and 0.69 for females).

Table 3 showed high similarity coefficient values in *O.niloticus* from Manzalla lake than those of *O.aureus* from the fish farm (0.85 and 0.69 for male and female, respectively). Similarity coefficients with 0.85 for both males and females were also observed in comparing *O.aureus* from Manzalla lake with *O.niloticus* from river Nile. Very high SC (1.00 and 0.92 for male and female, respectively) were detected in *O.niloticus* with *O.aureus* from Manzalla lake. SC were less in both species of the private fish farm (0.77 and 0.62 for male and female, respectively).

DISCUSSION

Chen and Tsuyuki (1970) and McKenzie (1973) mentioned that, sarcoplasmic proteins in many fishes are monomorphic and species specific, hence, used for evolution studies. Chen and Tsuyuki (1970) reported that sarcoplasmic electropherogram for mouth brooder species of tilapia (*Tilapia hornorum* and *T.mossambica*) are very similar, but differ from that of substratum spawner (*T.zillii* and *T.melanopleura*). The same authors also recorded that, muscle myogen pattern of F1 hybrid (*T.mossambica* O x *T.hornorum* O and Vice versa) have the characteristics of their parents, but they could not differentiate the genotype of the hybrid. Heines and Yashouv (1970) found significant variations in some muscle proteins between *T.aurea* collected from different locations, although morphological variations existed between these locations. Utter and Hodgins (1971) said that proteins of skeletal muscle are polymorphic in fish, and used them for studying the genetics of many species through electrophoretic analysis.

The muscle electrophoresis patterns showed polymorphism in 4 fractions in

Table 3. Similarity coefficients (S.C.) of the mobility of different sarcoplasmic protein fractions for both males and females of two *Oreochromis* species.

Comparison	P<	Fractions with significant mobility				Similarity Coefficient (S.C.)	
		Males		Females		Fraction #	Total
		Fraction #	Total	Fraction #	Total		
<i>Oreochromis niloticus</i> M X F	0.02	9	10	12	11	0.23	0.15
	0.01	1,7,10,11		1,2,5,7,9,10			
	0.001	2,3,4,5,6		3,4,6,11			
M X N	0.05	--	1	9	2	0.92	0.85
	0.02	--		7			
	0.01	1		--			
F X N	0.05	7	9	--	10	0.31	0.23
	0.02	10		9			
	0.01	11		1,3,7,12			
	0.001	1,2,3,4,5,6		4,6,8,10,11			
<i>Oreochromis aureus</i> M X F	0.05	1,6	2	--	4	0.85	0.69
	0.02	--		3			
	0.01	--		11			
	0.001	--		1,2			
<i>O. niloticus</i> X <i>O. aureus</i> o.n. (M) X O.a. (F)	0.05	6	2	--	4	0.85	0.69
	0.02	3		5			
	0.01	--		2,3			
	0.001	--		1			
O.a. (M) X O.n. (F)	0.05	--	2	7	11	0.31	0.15
	0.02	--		11			
	0.01	3,7,9,10		4,5,6,9,12			
	0.001	1,2,4,5,6		1,2,3,10			
O.a. (M) X O.n. (N)	0.05	2	9	4	2	0.85	0.85
	0.01	--		11			
	0.001	1		--			
o.a. (F) X o.n. (N)	0.05	--	4	3	3	0.69	0.77
	0.02	2,6		4			
	0.001	1,3		1			
o.a. (M) X o.n. (M)	0.01	--	-	11	1	1.00	0.92
O.n. (F) X O.a. (F)	0.05	9	3	4	5	0.77	0.62
	0.02	10		6			
	0.01	11		9,10,11			

M : Manzalla lake

F: Private fish farm

N : River Nile (Benha area)

O.n. : *Oreochromis niloticus*O.a. : *Oreochromis aureus*

case of male *Oreochromis niloticus* from Manzalla and private fish farm, and in 7 ones from river Nile. The polymorphism was in 5,3 and one fraction for female *O.niloticus* from Manzalla private fish farm and river Nile, respectively (Table 1). This may be attributed to the effect of locality and their genetic basis (Brody *et al.* 1976).

The similarity coefficient for both sexes of *O.niloticus* from Manzalla and river Nile is more than that from the private fish farm and the river Nile, and that from Manzalla and the private fish farm is the least one (0.92, 0.31 and 0.23 for males and 0.85, 0.23 and 0.15 for females, respectively), (Table 3). This indicates that the relative genetic purity is higher for *O.niloticus* from the river Nile, followed by those from Manzalla, but those from the private fish farm have the lowest one. This result agreed with that mentioned by Badawy (1993).

On the other hand, the muscle proteinogram of *O. aureus* from Manzalla lake and private fish farm showed similarity coefficient of 0.85 and 0.69 for males and females, respectively. This indicates that *O.aureus* from lake Manzalla (where the two species of *Oreochromis* are found) has more relative genetic purity than those reproduced and reared many times in polyculture with *O.niloticus* under pond conditions.

Furthermore, both sexes of *O.aureus* from Manzalla lake indicated higher similarity coefficient (0.85 for both sexes) than the same species from private fish farm (0.69 and 0.77 for males and females, respectively) when compared to both sexes of *O.niloticus* from the river Nile (Table 3). This sustains the above mentioned results because, in the natural opened body waters, the two sexes of each species prefer self spawning (intraspawning), and intercrossing between the sexes of different tilapia species is rare depending on their spawning habits and the surrounding conditions. The same case is found in closed pond conditions but the chance of hybridization (interspawning) is higher than that in open water bodies. For that reason, the crossing between the two species takes place only under a closed limited area (Payne and Collinson 1983 and Badawy 1993). So, it could be concluded that, the relative genetic purity of *O.niloticus* from the Nile is more than those from lake Manzalla, and those from the private fish farm have the least one. Also, *O.aureus* from lake Manzalla is more genetically pure than those from the private fish farm (Taniguishi *et al.* 1985).

Thus, it can be concluded that, in order to carry out crossing, *O.niloticus* and

O. aureus should have a relatively high genetic purities. This could be done by two ways: 1) by self spawning (intraspawning) of each species in completely separated ponds for many generations to purify each species genetically, or 2), in nature by selecting the broodstock of each species from water bodies which are empty of, or rarely contain the other species.

REFERENCES

1. Badawy, E.A. 1993. "Biological studies on Tilapia Species as a Major Component of the Egyptian Farming System". Thesis, Ph.D., Fac. Sci., Zagazig Univ.
2. Brody, T. R. Moav, Z.V. Abamson, G. Hulata and G. Wohlfarth. 1976. Application of electrophoretical genetic markers to fish breeding. II. Genetic variation within maternal half-sibs in carp. *Aquaculture*, 9 : 351-365 .
3. Chen, Y. F. and H. Tsuyuki. 1970. Zone electrophoretic studies on proteins of *Tilapia mossambica* and *Tilapia hornorum* and their hybrids. *Tilapia zillii* and *Tilapia melanopleura*. *J. Fish. Res. Bd.*, 27 : 2167-2177.
4. El-Saied, H.E. 1993. "Genetical Studies on Family Mugilidae in Two Different Habitats of Egyptian Waters". Thesis, M.Sc., Fac. Sci. (Benha), Zagazig Univ.
5. Ferguson, A. 1980. "Biochemical Systematic and Evolution". Glasgow, Blackie.
6. Heines, R. and A. Yashouv. 1970. Preliminary studies on muscle protein polymorphism occurring within the *Tilapia*. *Bamidgeh*, 22 : 69 .
7. Herzberg, A. and R. Pasteur. 1975. The identification of grey mullet species by disc electrophoresis. *Aquaculture*, 5:99-106.
8. McKenzie, J.A. 1973. Comparative electrophoresis of tissues from blue herring, *Alosa aestivalis* and caspareau *Alosa pseudoharingus*. *Comp. Biochem. Physiol.*, 44 B : 65-68 .
9. Payne, A.I. and R.I. Collinson. 1983. A comparison of biological characteristics of *Sarotherodon niloticus* (L.) with those of *Sarotherodon aureus* (Steindachner) and other tilapia of Delta and Lower Nile. *Aquaculture*, 30 : 335-351.

10. Payne, R.H., A.R. Child and A. Forrest. 1971. Geographical variation in the Atlantic salmon. *Nature*, 231: 250-252.
11. Rothbard, S. and Y. Pruginin. 1975. Induced spawning and artificial incubation of tilapia. *Aquaculture*, 5: 315-321.
12. Snedecor, G.W. and W.G. Cochran. 1976. "Statisticaal Methods". 6th Ed., Iowa state Univ. Press, Ames, Iowa.
13. Taniguishi, N., M.J. Macaranas and R.S. Pullins. 1985. Introgressive hybridization in cultured Tilapia stocks in Phillippines. *Bull. Jap. Soc. Sci. Fish.*, 51 (8) : 1219-1224.
14. Utter, F.N. and H.O. Hodgins. 1971. Biochemical polymorphisms on the Pacific hak (Merluccis products). *Rapp. Proc. Ver. Renu.*, 161: 87-89.
15. Wohlfarth, G.W. and G. Hulata. 1981. "The Applied Genetics of Tilapia". ICLARM Studies and Review. 6, 26, P. Manila, Phillippines.

دراسة مقارنة للفصل الكهربائي لبروتين عضلات نوعين من أسماك البلطي من مناطق مختلفة

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تم الفصل الكهربائى لعضلات ذكور واثاث سمكتى البلطى النيلى والاوريا المأخوذتين من بحيرة المنزله ونهر النيل (بنها) ومزرعه سمكيه خاصه (محافظة الشرقيه) بواسطه التحليل الكهربائى الجيلاتينين. وتم دراسة النسبيه المئويه لظهور الاجزاء المختلفه والحركه النسبيه لها بالاضافه الى معامل التماثل فى حركة الاجزاء المتماثله بين المجموعات المختلفه .

تم استبيان ١٢ جزءاً بنسب ظهور مختلفه لكلتا السمكتين منها ثلاثه أجزاء (رقم ٩ ، ١٠ ، ١٢) تم ظهورها فى كل العينات المدروسه.

تم تسجيل اختلافات فى الحركه النسبيه للاجزاء المختلفه مع بيان اختلاف معنوى بين الجنسين فى الجزء الاول من عضلات سمكه البلطى النيلى المأخوذة من نهر النيل والجزء الرابع من عضلات سمكه البلطى الاوريا المأخوذة من بحيرة المنزله.

تم حساب اعلى معامل تماثل فى حركه الاجزاء المتشابهه عند مقارنه كل من جنسى البلطى النيلى المأخوذة من بحيرة المنزله مع مثيلتها المأخوذة من نهر النيل، بينما اقل معامل تماثل وجد عند مقارنه اسماك البلطى النيلى المأخوذة من المزرعه الخاصه مع نفس النوع المأخوذ من بحيرة المنزله أو نهر النيل، مما يوضح أن اعلى نقاء وراثى نسبى (من وجهه النظر البيوكيميائيه) يكون موجوداً فى اسماك البلطى النيلى المأخوذة من نهر النيل ثم من بحيرة المنزله، أما الاسماك المأخوذة من المزرعه الخاصه فهو أقلها.

عند مقارنه نوعى السمك من نفس المنطقه وجد أن اعلى معامل تماثل فى كلا الجنسين المأخوذين من بحيرة المنزله، وأقلها من المزرعه السمكيه الخاصه.