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GENETIC VARIATION BETWEEN MALES AND FEMALES IN NILE PERCH (*Lates niloticus*) AS REVEALED BY RANDOM AMPLIFIED POLYMORPHIC DNA ASSAY

Mervat, M. Hashad; N.T. Hamdoon and E.A. Mohamed

Department of Genetics, Faculty of Agricultural, Assiut University Assiut-Egypt

Abstract: Nile perch (*Lates niloticus*) is one of the most important aquacultural species in Egypt. The goal of this work is to search for sex-specific DNA sequences in the male and female genomes of Nile perch by comparative random amplified polymorphic DNA (RAPD) assays performed on pooled DNA samples. Fifteen 10-mer arbitrary primers were used in RAPD-PCR analysis in this investigation. All the primers were amplified successfully on the genomic DNA from samples of males and females and yielded distinct RAPD pattern. The numbers of fragments amplified per primer varied between 3 (OPO-17) and 13 (OPO-16) and had a size ranged from 215 to 2060 bp. A total of 104 bands were amplified, and out of them, 7 bands (6.73%) were polymorphic while another 97 bands (93.27 %) were monomorphic. Primers OPA-11 and OPO-17 generated female specific bands, while primers OPO-2 and OPO-14 generated male specific bands. The primer OPE-1 generated two specific bands: the first is specific for males and the second is specific for females. The other primers failed to generate any sex- specific bands.

According to our knowledge these are the first sex-specific DNA markers isolated from a Nile perch fish species.

Key words: *Lates niloticus*, RAPD, Sex determination, Sex specific DNA markers.

Introduction

Little is known about sex determination and early sex differentiation in fish, despite the essential role of these processes in fish production. In most fishes species examined karyologically no morphologically differentiated sex chromosomes were found (Ohno, 1967). Therefore, mechanisms of sex determination have usually been inferred from genetic rather than cytological analysis. Genetic analysis of sex-linked molecular markers can discern differences in sex chromosomes at the nucleotide level (Clifton and Rodriguez, 1997). The number of sex-linked genes or markers found in fishes is low in comparison to more recent vertebrate groups, and most markers do not seem to be applicable to more than one fish species or even strain (Iturra *et al.*, 1998). Hadrys *et al.* (1992) suggested that sex-linked

markers may be easily identified, even in complex and relatively unknown genomes, by PCR amplification using single primers of random sequence composition, commonly referred to as RAPDs. Bardakci, (2000) showed that RAPD markers were successfully used in discrimination of sexes in Nile tilapia (*Oreochromis niloticus*). Two sex-linked RAPD markers were identified from the male DNA pool of the African catfish, *Clarias gariepinus* (Kovacs *et al*., 2001).

On the other hand, RPAD technique failed to detect a male specific genetic marker in Atlantic salmon, *Salmo salar* L. (McGowan and Davidson 1998). Furthermore, an extensive search done by Li *et al.* (2002) did not identify genomic sex markers in the green spotted pufferfish, *Tetraodon nigroviridis*. Moreover, similar results have been obtained by Yue *et al.* (2003) who performed RAPD analysis on male and female DNA pools of Asian arowana, *Scleropages formosus*. The comparative RAPD assays performed with 800 10-mer primers failed to yield a sex-associated marker.

The present study was conducted to investigate the possibility of using random amplified polymorphic DNA (RAPD) for developing sex-linked markers to be used in discriminating between both sexes.

Materials and Methods

Material:

Ten adult specimens of Nile perch (*Lates niloticus*) including 4 males and 6 females were obtained from Lake Nasser, Aswan, Egypt during summer 2004. Total length of the collected specimens ranged from 40 cm to 55 cm and their body weight ranged from 1860 g to 4700 g.

Methods:

1- RAPD-PCR Analysis:

Fifteen 10-mer arbitrary primers (Operon Tech., Inc) were used in RAPD-PCR analysis which their code numbers and sequences are shown in Table (1).

2- Genomic DNA isolation:

Male and female genomic DNA was isolated from liver tissues of four males and six females as two bulked samples. Liver tissue was transferred into liquid nitrogen and freezed until processed for RAPD analysis at Agricultural Genetic Engineering Research Institute (AGERI), Giza, Egypt. Genomic DNA was extracted by using Gentra Kit without modifications. Briefly, 100 mg of the liver tissue from each sex was cut into small pieces and suspended in 3 ml cell lyses solution. Seven and half µl proteinase K (20 mg/ml) was added and the mixture was incubated at 55°C for 2 - 3 hrs. Then 7.5 µl

proteinase K (20 mg/ml) was additionally added and incubation at the same temperature and the incubation was continued till no tissue aggregates appeared. Fifteen µl RNase A solution (4 mg/ml) was added to cell lysate and the mixture was incubated at 37°C for 30 min. One ml of protein precipitation solution (100 % Isopropanol) was added to the RNase A- treated cell lysate. DNA was precipitated with 100% Iso-propanol and then washed with 70% ethanol.

Table(1): Nucleotide sequences of the fifteen primers used to detect differences between males and females.

No.	Primer code No.	Nucleotide sequence (5´ to 3´)
1	OPA-11	CAATCGCCGT
2	OPO-1	GGCACGTAAG
3	OPE-1	CCCAAGGTCC
4	OPO-16	TCGGCGGTTC
5	OPO-17	GGCTTATGCC
6	OPO-14	AGCATGGCTC
7	OPO-18	CTCGCTATCC
8	OPO-4	AGGTCCGCTC
9	OPO-2	ACGTAGCGTC
10	OPO-8	CCTCCAGTGT
11	OPO-13	GTCAGAGTCC
12	OPO-6	CCACGGGAAG
13	OPO-9	TCCCACGCAA
14	OPO-11	GACAGGAGGT
15	OPO-I5	TGGCGTCCTT

3- Polymerase chain reaction (PCR) conditions:

The protocol described by Williams *et al.,* (1990) was employed for RAPD analysis with 15 primers. DNA amplification was carried out in a I Cycler (BIO-RAD).

4- PCR program:

The thermal cycles used were: one cycle of 5 min at 94°C, then 40 cycles of 60 sec at 94 ºC, 60 sec at 36 ºC., 90 sec at 72 ºC and a final extension cycle 72 ºC for 5 min.

5- Electrophoresis:

Ten µL from each sample was analyzed in 1.7% agarose gel prepared in 1X TAE buffer. The gel

was stained with ethidium bromide (1%) and photographed under Ultraviolet light. Hundred Base pair ladder (Amercham Biosciences) was used as standard DNA marker.

6- Data analysis:

Agarose gel photos were scanned by Gene profiler 4.03 computer software program that uses automatic lane and peak finding to detect the presence of bands in a gel, and calibrate them for size and density. A binary data matrix containing the presence (1) or absence (0) of bands was made.

Results

All primers were amplified successfully on the genomic DNA extracted from male and female samples of *Lates niloticus*. The DNA fragments generated by the 15 primers from the genomic DNA of male and female samples of *L. niloticus* were separated using Agarose gel electrophoresis and illustrated in Figs. 1 and 2. The banding patterns of these DNA fragments were summarized in Tables (2, 3 and 4).

Data in Table (2) showed that the number of fragments amplified per primer varied between 3 (OPO-17) and 13 (OPO-16) bands with an average of 6.9 bands/primer. The size of the amplified fragments also varied with different primers. The size of fragments ranged from 215 bp (OPO-6) to 2060 bp (OPO-16). The tested primers generated 101 bands in females and 100 bands in males of *L. niloticus* (Table 3). A total of 104 DNA bands were generated by the 15 primers for males and females of *L. niloticus*. Out of these DNA bands, 97 (93.27 %) were conserved between males and females while 7 bands (6.73 %) were polymorphic. Only 5 out of the 15 tested primers produced polymorphism between males and females of *L. niloticus* (Table 3). These primers were, OPA-11 (16.67 % polymorphism), OPE-1 (25 %), OPO-17 (66.67 %), OPO-14 (20 %) and OPO-2 (14.29 %). The two primers of OPA-11 and OPO-17 generated female specific bands. In contrast, the two primers OPO-14 and OPO-2 generated male specific bands. While No sex-specific bands were detected by the rest primers. The results in Table (4) also showed that four bands 1880 (OPA-11), 255 (OPE-1), 1530 and 1260 bp (OPO-17) were specific markers for females. While 1065 (OPE-1), 425 (OPO-14) and 695 bp (OPO-2) were unique molecular markers for males *L. niloticus*.

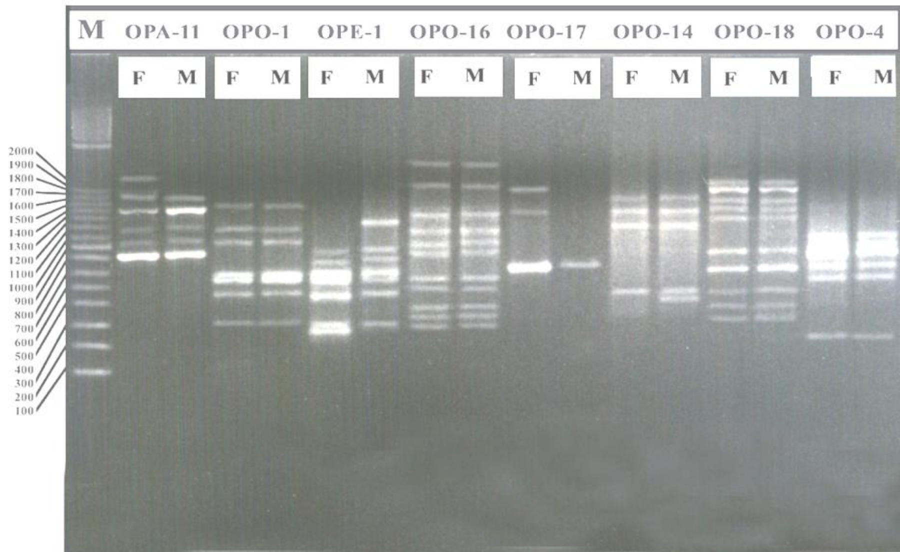

Figure (1): Agarose-gel electrophoresis of RAPD products generated with primers (OPA-11), (OPO-1), (OPE-1), (OPO-16), (OPO-17), (OPO-14), (OPO-18) and (OPO-4) in F = females and M = males of *Lates niloticus.*

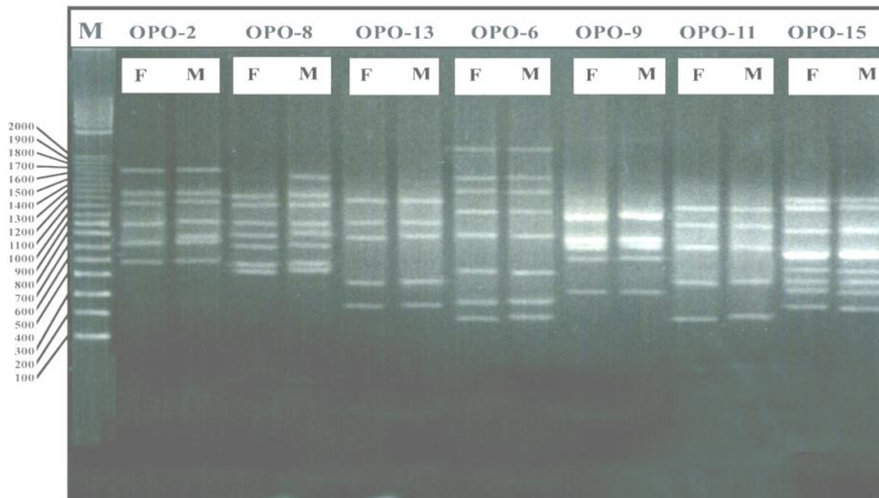

Figure (2): Agarose-gel electrophoresis of RAPD products generated with primers (OPO-2), (OPO-8), (OPO-13), (OPO-6), (OPO-9), (OPO-11) and (OPO-15) in F = females and M = males of *Lates niloticus.*

Table(2): Survey of amplified RAPD fragments using 15 primers in females and males of *Lates niloticus* where(1) means presence and (0) means absence.

Band No.	Primer	Band size pb	Female	Male	Band No.	Primer	Band size pb	Female	Male
1	OPA-11	1880	1	0	53	OPO-4	900	1	1
2		1465	1	1	54		825	1	1
3		1195	1	1	55		755	1	1
4		975	1	1	56		635	1	1
5		840	1	1	57		555	1	1
6		715	1	1	58		235	1	1
7	OPO-1	1260	1	1	59	OPO-2	1535	1	1
8		980	1	1	60		1175	1	1
9		840	1	1	61		1025	1	1
10		580	1	1	62		810	1	1
11		540	1	1	63		695	0	1
12		455	1	1	64		635	1	1
13		300	1	1	65		500	1	1
14	OPE-1	1065	0	1	66	OPO-8	1420	1	1
15		780	1	1	67		1165	1	1
16		690	1	1	68		1025	1	1
17		590	1	1	69		815	1	1
18		555	1	1	70		740	1	1
19		455	1	1	71		625	1	1
20		285	1	1	72		480	1	1
21		255	1	0	73		445	1	1
22	OPO-16	2060	1	1	74	OPO-13	1095	1	1
23		1590	1	1	75		855	1	1
24		1235	1	1	76		710	1	1
25		1150	1	1	77		380	1	1
26		1080	1	1	78		265	1	1
27		955	1	1	79	OPO-6	1920	1	1
28		845	1	1	80		1435	1	1
29		750	1	1	81		1220	1	1
30		545	1	1	82		950	1	1
31		480	1	1	83		705	1	1
32		380	1	1	84		440	1	1
33		330	1	1	85		285	1	1
34		280	1	1	86		215	1	1
35	OPO-17	1530	1	0	87	OPO-9	935	1	1
36		1260	1	0	88		695	1	1
37		635	1	1	89		635	1	1
38	OPO-14	1390	1	1	90		545	1	1
39		1200	1	1	91		320	1	1
40		1010	1	1	92	OPO-11	1020	1	1
41		455	1	1	93		815	1	1
42		425	0	1	94		620	1	1
43	OPO-18	1650	1	1	95		395	1	1
44		1515	1	1	96		220	1	1
45		1335	1	1	97	OPO-15	1175	1	1
46		1230	1	1	98		1050	1	1
47		1080	1	1	99		790	1	1
48		770	1	1	100		585	1	1
49		605	1	1	101		485	1	1
50		460	1	1	102		400	1	1
51		395	1	1	103		355	1	1
52		330	1	1	104		280	1	1

Table(3): Number of amplified and polymorphic DNA-fragments in females and males of *Lates niloticus*.

No.	*Primer code*	*No. of amplified bands*		*Total of amplified bands*	*No. of polymorphic bands*	*Polymorphism %*
		Female	*Male*			
1	*OPA-11*	*6*	*5*	*6*	*1*	*16.67*
2	*OPO-1*	*7*	*7*	*7*	*0*	*0.0*
3	*OPE-1*	*7*	*7*	*8*	*2*	*25*
4	*OPO-16*	*13*	*13*	*13*	*0*	*0.0*
5	*OPO-17*	*3*	*1*	*3*	*2*	*66.67*
6	*OPO-14*	*4*	*5*	*5*	*1*	*20*
7	*OPO-18*	*10*	*10*	*10*	*0*	*0.0*
8	*OPO-4*	*6*	*6*	*6*	*0*	*0.0*
9	*OPO-2*	*6*	*7*	*7*	*1*	*14.29*
10	*OPO-8*	*8*	*8*	*8*	*0*	*0.0*
11	*OPO-13*	*5*	*5*	*5*	*0*	*0.0*
12	*OPO-6*	*8*	*8*	*8*	*0*	*0.0*
13	*OPO-9*	*5*	*5*	*5*	*0*	*0.0*
14	*OPO-11*	*5*	*5*	*5*	*0*	*0.0*
15	*OPO-I5*	*8*	*8*	*8*	*0*	*0.0*
Total		*101*	*100*	*104*	*7*	*6.73*

Table(4): Size of specific bands generated by different primers:

No.	*Primer code*	*Size of specific band (bp)*	
		Female	*Male*
1	*OPA-11*	*1880 bp*	---
2	*OPO-1*	---	---
3	*OPE-1*	--- *255 bp*	*1065 bp* ---
4	*OPO-16*	---	---
5	*OPO-17*	*1530 bp* *1260 bp*	--- ---
6	*OPO-14*	---	*425 bp*
7	*OPO-18*	---	---
8	*OPO-4*	---	---
9	*OPO-2*	---	*695 bp*
10	*OPO-8*	---	---
11	*OPO-13*	---	---
12	*OPO-6*	---	---
13	*OPO-9*	---	---
14	*OPO-11*	---	---
15	*OPO-I5*	---	---

Discussion

In the present investigation two bulked samples from both males and females of *Lates niloticus* were screened with 15 RAPD primers to detect sex-associated DNA markers. Three male-specific RAPD markers (1065_{OPE-1}, 425_{OPO-14} and 695_{OPO-2} pb) as well as four female-specific markers (1880_{OPA-11}, 255_{OPE-1}, 1530_{OPO-17} and 1260_{OPO-17} pb) were detected. The usefulness of these markers could be further improved by converting them into sequence-tagged-sites PCR (STS-PCR) markers as RAPD is rather sensitive to reaction conditions (Yue *et al.*, 2003). One approach for sex determination in fish, that has been successful with both plants (Hormaza *et al.*, 1994) and birds (Griffiths and Tiwari, 1993; Sabo *et al.,* 1994), is to screen for a specific markers using randomly amplified polymorphic DNAs (RAPDs) (McGowan and Davidson, 1998; Bardakci, 2000 and Hamdoon, 2003). Bulked segregant analysis can be performed on separate pools of DNA sampled from several males and several females in order to minimize the effects of individual variation and improve the efficiency of the screening process (Michelmore *et al.,* 1991). However, the number of sex-linked genes or markers found in fishes is low in comparison to more recent vertebrate groups, and most markers do not seem to be applicable to more than one fish species or even strain (Iturra *et al.*, 1998).

Obtaining a marker linked to a gene or genomic region through RAPD analysis depends to a large extent on chance because random sequences are used PCR primers (Bardakci, 2000).

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الاختلافات الوراثية بين ذكور و اناث اسماك قشر البياض من خلال تحليل الدنا المتعاظم عشوائيا

مرفت محمد حشاد، نصر الدين ثابت حمدون و السيد عبد المنصف محمد

قسم الوراثة- كلية الزراعة- جامعة اسيوط-أسيوط-مصر

تعد اسماك قشر البياض واحدة من اهم الانواع السمكية الموجودة في مصر وقد استهدفت هذه الدراسة البحث عن تتابعات الـ DNA الخاصة بجنس معين في جينوم الذكور او الاناث من خلال مقارنة طرز الدنا المتعاظم عشوائيا المشتقة من عينات الـ DNA المجمعة من كل من الذكور و الاناث حيث يعتمد هذا التكنيك علي تفاعل البلمرة المتسلسل الذي يستخدم بادئات تقوم بتكبير او تعظيم مناطق محددة من الجينوم.

تم الحصول علي طرز حزمية مميزة لكل بادئ مع كل من الذكور والإناث، وقد إختلف عدد الشظايا الناتجة لكل بادئ من 3-13 بمتوسط 6.94 حزمة لكل بادئ وتراوحت أحجام شظايا الدنا المتعدد المظاهر المتعاظم عشوائيا بين 215-2060 زوج من القواعد. وقد أظهرت 7 حزم (بنسبة 6.73%) من 104حزمة تعددا مظهريا ووجدت 97 حزمة مشتركة بين الذكور و الاناث في العينات المدروسة. وقد أظهر البادئ OPO-16 أكبر عدد من حزم الدنا (13 حزمة) في الوقت الذي أظهر فيه البادئ OPO-17 أقل عدد من هذه الحزم (3 حزمة). وقد أعطي البادئ OPA-11 حزمة واحدة مميزة للاناث بينما أعطي البادئ OPO-17 حزمتين مميزتين للإناث. و اعطي كل من البادئان OPO-2 وOPO-14 حزمة واحدة مميزة للذكور. وقد أعطي البادئ OPE-1 حزمة واحدة مميزة للذكور و أخري مميزة للإناث. بينما لم تظهر باقي البادئات اي حزم مميزة للذكور عن الاناث.