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The Use of Random Amplified Polymorphic DNA (RAPD) Markers in Sex Discrimination in Nile Tilapia, *Oreochromis niloticus* (Pisces: Cichlidae)

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Abstract: Random amplified polymorphic DNA (RAPD) markers were successfully used in discrimination of sexes in Nile tilapia fish (*Oreochromis niloticus*) using linear discriminant function analysis. The results provide support for the view that major genetical sex determining factors exist in tilapia.

Key Words: RAPD, DNA, sex discrimination, tilapia, *Oreochromis*

Nil Tilapia Balığının (Pisces: Cichlidae) Cinsiyet Ayırımında Rastgele Çoğaltılmış Polimorfik DNA (RAPD) Markerlerinin Kullanımı

Özet: Rastgele çoğaltılmış polimorfik DNA (RAPD) markerleri kullanarak lineer ayrıcı fonksiyon analizi, Nil tilapia balığının (*Oreochromis niloticus*) cinsiyetlerini ayırmada başarılı olarak kullanıldı. Sonuçlar tilapiada ana genetiksel cinsiyet belirleme faktörlerinin olduğu görüşüne destek sağlamaktadır.

Anahtar Sözcükler: RAPD, DNA, cinsiyet ayrımı, tilapia, *Oreochromis*

Introduction

Amongst vertebrates, fishes contain the greatest variability in sex determination mechanisms including monofactorial, polyfactorial and environmental control (1). In most cases, genes located on the heteromorphic sex chromosomes play the main role in the expression of sex determination in fishes (2). On the other hand, morphological differentiation of sex chromosomes is not apparent in most fish species studied, thus has been demonstrated cytogenetically in few fish species (3-5). The most recent approach to studying the mechanism of sex determination in fishes is to develop sex-specific molecular markers. So far sex-specific markers have only been developed in the guppy, *Poecilia reticulata* (6), chinook salmon, *Oncorhyncus tshawytscha* (7), coho salmon, *O. kisutch* (8) and *Leporinus elongatus* (9).

Studies of the sex determination mechanism in tilapia are primarily based on the sex ratio of offspring obtained from inter- and intra-specific crosses, crosses between sex reversed parents and after chromosome manipulations leading to polyploid, gynogenetic and androgenetic individuals, as well as cytogenetic methods, and several hypotheses, including monofactorial, polyfactorial, autosomal and environmental sex determination, have been proposed (10-12). In

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addition, several studies were carried out to identify heteromorphic sex chromosomes, but without success (13-15). Hybridization with known sex-linked nucleic acid sequences and subtractive hybridization have also so far failed in the detection of sex-specific DNA markers in tilapia (16).

The objective of the present study was to use the Random Amplified Polymorphic DNA (RAPD) method (17) to develop sex-linked RAPD markers and to use them in discriminating between sexes of *Oreochromis niloticus niloticus.*

Materials and Methods

The manzala strain used in this study is known to be relatively inbred in comparison with other strains of this species (18). Three different genotypes (referred to as XX female, XY male and YY male) were used. These were chosen on the basis of the known sex ratios obtained in progeny tests (19). YY male fish generally produce 100% male offspring in crosses with XX females and XY sex-reversed females. These three genotypes are referred to for convenience as genotypic sexes. This does not imply that specific sex chromosomes exist in this species. All YY males and XY females were from the crosses, (1) XY sex-reversed female x YY male and (2) XY male x XX female, respectively. The male parent of cross (2) was an offspring from cross (1). XY males used in the RAPD experiment were progenies from both crosses.

The isolation of DNA from fin tissue samples, and conditions for the amplification of RAPD markers and non-denaturing polyacrylamide gel electrophoresis are described by Bardakci and Skibinski (18). Although RAPD markers were resolved mainly using 5% non-denaturing polyacrylamide gel electrophoresis, most of them were also separated on 1.5% agarose gels in 1xTAE buffer (0.4M tris, 5mM sodium acetate and 1mM disodium EDTA pH 8.2) visualized with ethidium bromide in order to simplify the profile of RAPD bands.

Results

Initially, bulk segregant analysis (20) was used to search for sex-specific markers with a total of 140 10-mer RAPD primers (OPA, OPB, OPC, OPD, OPE, OPF and OPG, each containing 20 primers) from Operon Tech. Alameda, CA. Comparison of amplification of pooled DNA samples from three genotypic sexes failed to show any reproducible and clear cut RAPD markers occurring in one sex alone. Therefore, the first three sets of primers (sets OPA, OPB and OPC) were tested on four individuals of each genotypic sex separately. Of these primers, 21 primers produced polymorphism between sexes. A total of 173 bands were examined and each primer included in the analysis produced 8 bands on average. Of these, 46 bands were polymorphic between individuals tested.

As three genotypic sexes were analyzed, the potential existed for the identification of both X and Y chromosome-linked RAPD markers. In the first instance, data were examined for bands that were unique to individuals carrying X or Y. Any marker linked to the Y would be present in both YY and XY males. Similarly, X markers were expected to be present in both XX females and

XY males. Results showed no single, reproducible RAPD band unique to individuals with X or Y. Therefore all gels were scored for presence (1) and absence (0) with the aim of using linear discriminant function analysis (SPSS Inc.) to identify a combination of bands that might discriminate between the genotypic sexes. RAPD fragment patterns of two primers are shown in Fig. 1a,b.

The results showed significant difference (P=0.004; Wilks' lambda=0.006) between individuals with XX and XY genotypic sex and individuals with YY genotypic sex. In addition the difference between individuals with and without Y was significant (P= 0.004; Wilks' lambda= 0.006). The individuals used for analysis were obtained from two closely related families (see above). The analysis failed to discriminate between families (P=0.676; Wilks' lambda=0.222) giving confidence that significant X versus Y related differences are not the result of family genetic differences unrelated to sex. When three genotypic sexes were compared in the same test, no significant discrimination between them was obtained (P=0.550; Wilks' lambda 0.252). Large values of Wilks' lambda suggest that group mean scores tend not to be different.

Results of canonical discriminant function analysis showed correct classification (100%) of all individuals within group defined Y and X. The significant discrimination between individuals belonging to three different genotypic sexes was the result of the intermediacy of XY - the differences in discriminant scores are highest between the YY and XY genotypic sexes. Discriminant function scores are given in Table 1.

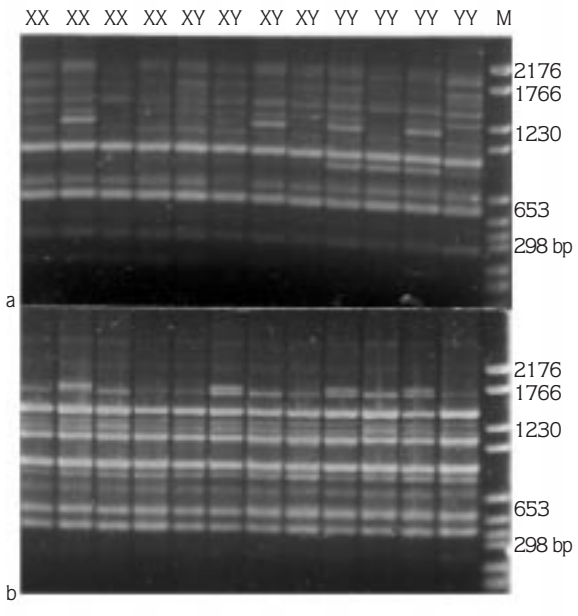

Figure 1. RAPD fragment patterns of primers (a) OPA02 and (b) OPA07 from three genotypic sexes of *O. niloticus*. M: Molecular weight marker.

Table 1. Discriminant function scores of RAPD markers. Contrasting groups of values are given in bold and normal font.

	Discriminant function scores			
Genotypic sexes	Group Y	Group X	Genotype	Family
YY	**7.922**	**16.247**	**11.349**	**0.732**
YY	**8.728**	**17.053**	**12.113**	**0.732**
YY	**7.519**	**15.845**	**10.962**	**2.196**
YY	**8.728**	**17.053**	**12.105**	**3.660**
XY	**9.131**	-7.519	1.028	**2.196**
XY	**7.922**	-8.728	-0.123	**0.732**
XY	**9.131**	-7.519	1.028	-2.196
XY	**7.117**	-9.534	-0.888	-0.792
XX	-17.053	-8.728	**-12.369**	-0.732
XX	-15.845	-7.519	**-11.226**	-2.196
XX	-18.262	-9.936	**-13.520**	-2.196
XX	-15.039	-6.714	**-10.461**	-2.196

One of the important points arising from the present study was to select markers for effective separation of sexes. Stepwise discriminant function analysis was used to achieve this objective. As a result, 6 RAPD primers (OPA11, OPA02, OPB08, OPC14, OPC18 and OPC11) giving the best discrimination between individuals with and without Y were selected. Similarly, 5 RAPD primers (OPA02, OPA04, OPA11, OPB18 and OPA13) were selected that discriminated individuals with and without X.

Discussion

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 as PCR primers. For example, although Levin et al. (21) obtained 13 Z-linked RAPD markers in chickens using only 298 primers, Hormaza et al. (22) found a single female specific RAPD marker in pistachio, *Pistacia vera* using 700 primers. Moreover, the chance of any RAPD markers being linked to a gene or a genomic region of interest is dependent on genome size, type of gene or genomic region (dominant or codominant) and on the type of population used to generate markers. Genetic homogeneity between groups compared (apart from in the target genomic region) will increase

the chance of detection of a marker linked to the target region. The subspecies used in this study is relatively inbred in comparison with other subspecies and strains of this species (18) and this should have favoured identification of markers linked to a segregating sex determining genomic region. One reason for the failure to obtain perfectly reproducible clear cut differences could be the complexity of the mechanism of sex determination in tilapia. For example, Mair et al. (23) obtained a small proportion of males from several *O. niloticus* gynogens. These males were progenies tested and found to be naturally sex-reversed females with XX genotypes. Several hypotheses have been proposed for sex determination in tilapia based on studies of interspecific hybridizations, chromosome set manipulations, sex inversion and intraspecific crosses (10-12). However, none of these hypotheses gives a satisfactory explanation for all data. A plausible model is that sex in tilapia is controlled by major sex determining factors with aberrant sex ratios being the result of minor sex modifying factors. Recently, Baroiller et al. (24) demonstrated the effect of high temperature on the sex ratio of *O. niloticus*. The result of the present study also give support to the hypothesis of the presence of a major sex determining factor in *O. niloticus.* This is simply because it has proved possible to discriminate between the X and Y groups. However, the observation that several RAPD bands are necessary for the perfect discrimination suggests that minor sex factors associated with RAPD markers might also be involved. Consequently, regarding sex as a quantitative trait appears to be the most hopeful approach in sex determination in tilapia.

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References

1. Bull J.J., Evolution of sex determining mechanisms. Benjamin/Cummings Inc., Menlo Park, CA, 1983.
2. Kirpichnikov V.S., Genetic Basis of fish selection. Springer-Verlag, Heidelberg, 1981
3. Thorgaard G.H., Heteromorphic sex chromosomes in male rainbow trout. Science 196, 900-902, 1977.
4. Lloyd M.A. and Thorgaard G.H., Restriction endonuclease banding of rainbow trout chromosomes. Chromosoma 96, 171-177, 1988.
5. Reed K.M. and Phillips R.B., Molecular characterization and cytogenetic analysis of highly repeated DNAs of lake trout, Salvelinus namaycush. Chromosoma 104, 242-251, 1995.
6. Nanda I.W., Feichtinger W., Schmid M., Schroeder J.H., Zischler H. and Epplen J.T. Simple repetitive sequences are associated with differentiation of the sex chromosomes in the guppy fish. J. Mol. Evol. 30, 456-462, 1990.

7. Devlin R.H., McNeil B.K., Groves, T.D.D., Donaldson, E.M., Isolation of a Y-chromosomal DNA probe capable of determining genetic sex in chinook salmon (*Oncorhynchus tshawytscha*). Can. J. Fish. Aquat. Sci. 48, 1606-1612, 1991.

8. Forbes S.H., Knudson K.L., North T.W. and Allendorf F.W., One of two growth hormone genes in coho salmon is sex-linked. Proc. Natl. Acad. Sci. USA 91, 1628-1631, 1994.

9. Nakayama I., Foresti F., Tewari R., Schartl M. and Chourrout D., Sex chromosome polymorphism and heterogametic males revealed by two cloned DNA probes in the ZW/ZZ fish *Lepomis elongatus,* Chromosoma, 103, 31-39, 1994.

10. Wohlfarth, G.W. and Wedekind, H., The heredity of sex determination in tilapias. Aquaculture, 92, 143-156, 1991.

11. Mair, G.C., Chromosome-set manipulation in tilapia- techniques, problems and prospects. Aquaculture, 111, 227-244, 1993.

12. Trombka, D. and Avtalion, R., Sex determination in tilapia - a review. The Israeli Journal of Aquaculture-Bamidgeh, 45, 26-37, 1993.

13. Kornfield, I.L., Descriptive genetics of Cichlid fishes. In Evolutionary Genetics of Fishes (ed. by B.J. Turner), New York, 1984, Plenum Publ. Corp., pp. 519-516.

14. Majumdar, K.C. and McAndrew, B.J., Relative DNA content of somatic cell nuclei and chromosomal studies in three genera, *Tilapia, Sarotherodon* and *Oreochromis* of the tribe Tilapiini. Genetica, 68, 165-168, 1986.

15. Crosetti, D., Sola, L., Brunner, P. and Cataudella, S. (1988). Cytogenetical characterization of *O. niloticus* and *O. mossambicus* and their hybrid. In 2nd Int. Symp. on Tilapia in Aquaculture (ed. by R.S.V. Pullin, T. Bhukaswan, K. Tanguthai, and J.L. Maclean), 1988, ICLARM Conf Proc., pp. 143-151.

16. McConnel, S.K.J., Studies on the Molecular Biology of Sex Determination in the Tilapias. PhD. Thesis. University of Wales, Swansea, 1993.

17. Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A. and Tingey, S.V., DNA polymorphisms by arbitrary primers are useful as genetic markers. Nucleic Acids Res., 18, 6531-6535, 1990.

18. Bardakci, F. and Skibinski, D.O.F., Applications of the RAPD technique in tilapia fish: species and subspecies identification. Heredity, 73, 117-123, 1994.

19. Mair, G.C., Penman, D.J., Scott, A., Skibinski, D.O.F. and Beardmore, J.A. , Hormonal sex-reversal and the mechanism of sex determination in *Oreochromis*. In Proc. World Symp. on Hybridization and Genetic Engineering in Aquaculture (ed. by K. Tiews), 1987, Bordeaux, France, pp. 301-312.

20. Michelmore R.W., Paran I. and Kesseli R.V., Identification of markers linked to disease- resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregating populations. Proc. Natl. Acad. Sci. 88, 9828-9832, 1991.

21. Levin, I., Crittenden, L.B., and Dodgson, J.B., Genetic map of the chicken Z chromosome using random amplified polymorphic DNA (RAPD) markers. Genomics, 16, 224-230, 1993.

22. Hormaza, J.I., Dollo, L. and Polito, V.S., Identification of a RAPD marker linked to sex determination in Pistacia vera, using bulked segregant analysis. Theor. Appl. Genet., 89, 9-13, 1994.

23. Mair, G.C., Scott, A.G., Penman, D.J., Beardmore, J.A. and Skibinski, D.O.F., Sex determination in the genus Oreochromis. 1. sex reversal, gynogenesis and triploidy in *O. niloticus* (L.). Theor. Appl. Genet., 82, 144-152, 1991.

24. Baroiller J.F., Clota F. and Geraz E. (1995). Temperature sex determination in two tilapia, *Oreochromis niloticus* and red tilapia (red Florida strain): effect of high or low temperature. In: Proc. of the fifth int. symp. on the reproductive physiology of fish (ed. by F.W. Goetz and P Thomas), Austin TX, 1995, pp. 158-160.