RAPD analysis in gynogenetic individuals in turbot (*Scophthalmus maximus*)

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Introduction

The turbot (*Scophthalmus maximus*) is one of the marine species with major commercial value in Europe. Despite this fact, the mechanism of sex determination is unknown. In our laboratory we have tried to develop a study in order to reveal genetic differences in both sexes. We have used a method for random amplified polymorphic DNA (RAPD), with genomic DNA from males and females of gynogenetic animals

Material and Methods

Fishes were supplied by the Oceanographic Institute of Vigo. Genomic DNA was extracted by the method of phenol-chloroform (Sambrook et al., 1989). For the isolation of a potential sex-linked molecular marker 560 primers were used with gynogenetic individuals, comprising A(1-20) to Z(1-20). All primers are 10mers. For this primers a pool of 5 males and a pool of 5 females were used. Analysis were performed with two concentrations of DNA: 20 ng/ml and 5 ng/ml. Amplification reactions were carried out in 25 ml (14.75 ml milliQ H_2O; 1X reaction buffer; 2 mM $MgCl_2$; 10 mM dNTP's - Amersham Biosciences - ; 0.2 mM primer – Sigma Genosys - ; 0.8 ng and 0.2 ng DNA, respectively; 1 U Taq DNA polymerase - Bioline -). Amplification was in a thermocycler for 1 cycle at 94 ºC for 2 min., 35 cycles at 95 ºC for 15 sec., 34 ºC for 45 sec., 72 ºC for 2 min. and 1 cycle at 72 ºC for 5 min. Electrophoresis of reaction products was carried out using 2% agarose gel and 1x TBE buffer containing 0.5 mg/ml ethidium bromide (Sambrook et al., 1989) for 5 about hours at 70 V. Band profiles visualized using a TFX-20.M transiluminator.

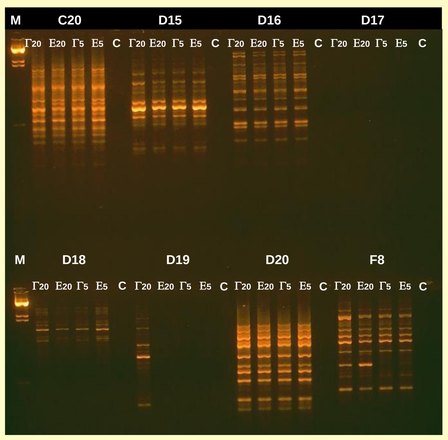

Fig. 1 – Digitalizated image of a RAPD amplification using the primers C20, D15-D20, F8 . M: Size marker (Lambda DNA/*HindIII*). C: negative control. Γ_{20}: DNA (20 ng/ml) from a pool of 5 males. E_{20}: The same for females. Γ_5: DNA (5 ng/ml) from a pool of 5 males. E_5: The same for females

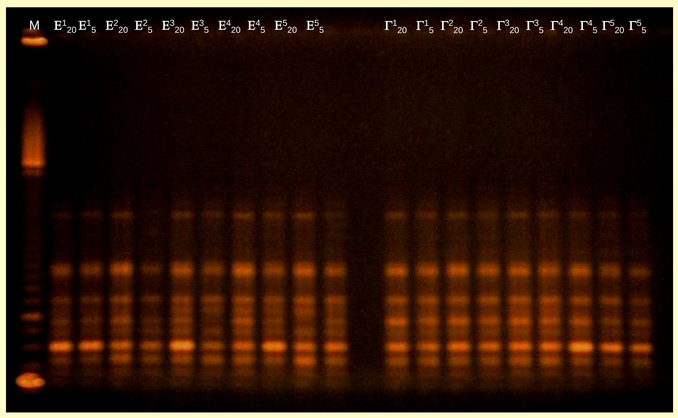

Fig. 3 - Digitalizated image of a RAPD amplification using the primer C20 in each individual of the pool. M: Size marker (Lambda DNA/*HindIII*). C: negative control. Γ_{20}: DNA (20 ng/ml). E_{20}: The same for females. Γ_5: DNA (5 ng/ml) E_5: The same for females. Superindex indicate different individuals

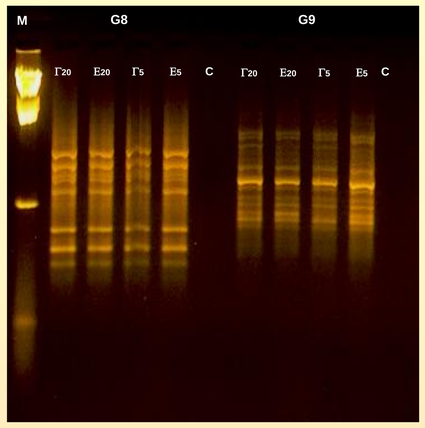

Fig. 2 - Digitalizated image of a RAPD amplification using the primers G8 and G9. M: Size marker (Lambda DNA/*HindIII*). C: negative control. Γ_{20}: DNA (20 ng/ml) from a pool of 5 males. E_{20}: The same for females. Γ_5: DNA (5 ng/ml) from a pool of 5 males. E_5: The same for females.

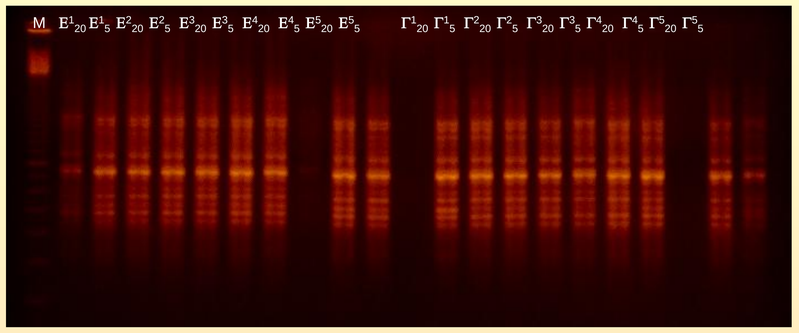

Fig. 4 - Digitalizated image of a RAPD amplification using the primer G9 in each individual of the pool. M: Size marker (Lambda DNA/*HindIII*). C: negative control. Γ_{20}: DNA (20 ng/ml) E_{20}: The same for females. Γ_5: DNA (5 ng/ml) E_5: The same for females. Superindex indicate different individuals.

Conclusion

We have used 560 primers in pooled DNA samples of gynogenetic males and females of *Scophthalmus maximus*. We have found three polymorphic bands. The analysis with individual samples could not confirm the existence of a definitive molecular marker for sex. Now we are evaluating the relevance of these sequences in turbot sex determination by isolating, cloning and sequencing them.

Results

The DNA of gynogenetic turbot was amplified with 560 primers, more than 90 % of which produced amplification products. The great majority of reactions resulted in monomorphic banding patterns, demonstrating the high genomic similarity between males and females of these gynogenetic fishes. With primer C20 (5'-ACTTCGCCAC-3') we obtained an amplification fragment of 400-500 bp in size (Fig. 1) which was related to an exclusive band present in the sample from female pool , this being repeated when the analysis was carried out with each individual of both pools (Fig. 3).

Primer G9 (5'-CTGACGTCAC-3') amplified a fragment of 1200 bp in male pool (Fig. 2). Samples of individual fishes shown that this difference between males and females was maintained in a high percentage (Fig. 4).

Finally, we amplified a fragment of 4300 bp in size with the primer F8 (5'-GGGATATCGG-3') (Fig. 1). This fragment did not appear when the analysis was carried out with each individual of the pool (data not shown).

References:

Sambrook, J., T. Maniatis and E.F. Fritsch, 1989. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, New York.