PRELIMINARY LINKAGE MAP IN TURBOT (*Scophthalmus maximus*) WITH AFLPs AND MICROSATELLITE MARKERS

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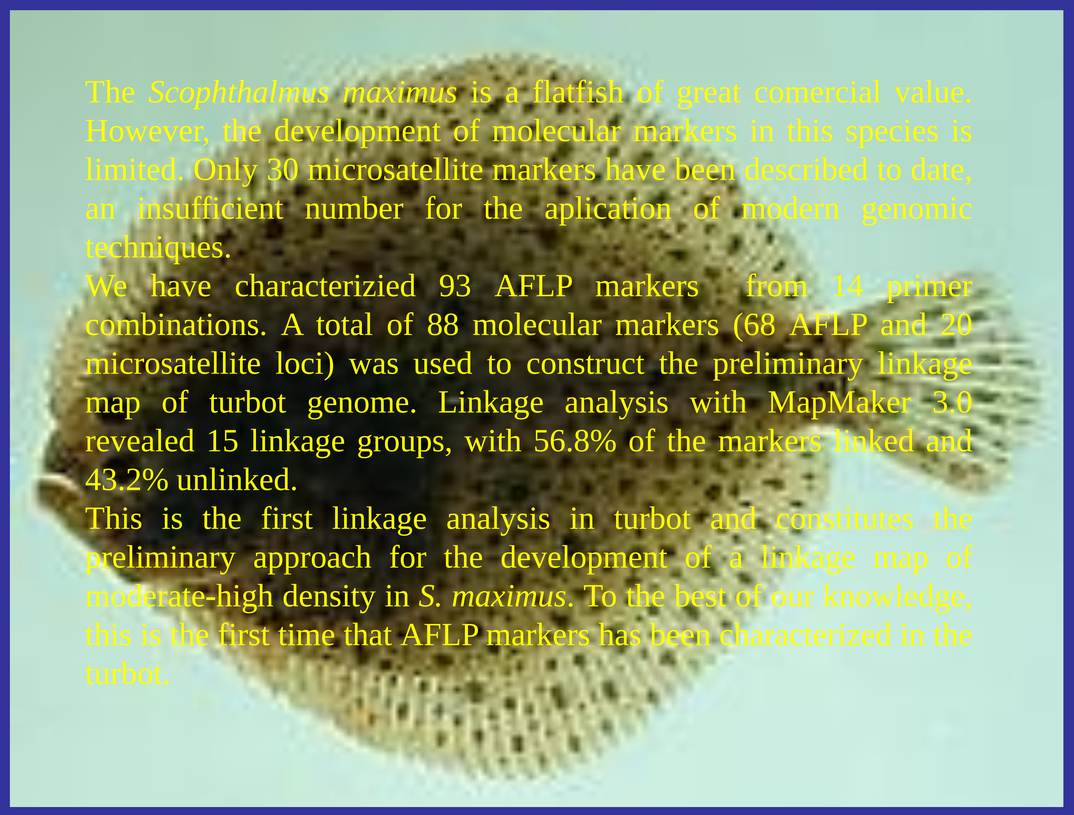
Introduction

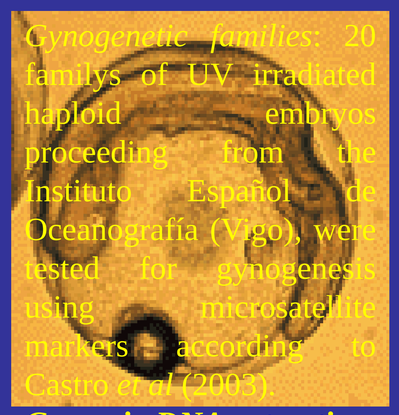


The *Scophthalmus maximus* is a flatfish of great comercial value. However, the development of molecular markers in this species is limited. Only 30 microsatellite markers have been described to date, an insufficient number for the aplication of modern genomic techniques.

We have characterizied 93 AFLP markers from 14 primer combinations. A total of 88 molecular markers (68 AFLP and 20 microsatellite loci) was used to construct the preliminary linkage map of turbot genome. Linkage analysis with MapMaker 3.0 revealed 15 linkage groups, with 56.8% of the markers linked and 43.2% unlinked.

This is the first linkage analysis in turbot and constitutes the preliminary approach for the development of a linkage map of moderate-high density in *S. maximus*. To the best of our knowledge, this is the first time that AFLP markers has been characterized in the turbot.

Material and Methods



Gynogenetic families: 20 familys of UV irradiated haploid embryos proceeding from the Instituto Español de Oceanografía (Vigo), were tested for gynogenesis using microsatellite markers according to Castro *et al* (2003).

Genomic DNA extraction: DNA was extracted following conventional phenol-clorophorm protocols . An overnigth digestion with a high concentration of PK was required to disolve the corion envelope

AFLP analysis: AFLPs were performed using the protocol by Razzoli *et al* (2003). Genomic DNA (about 150-200ng) was digested with *Eco*RI and *Taq*I. After ligation, 50ng of DNA were preamplified using primers with one selective base. For amplification, 20 primer combinations with three selective nucleotides were applied in 20 haploid individuals (Figure 1). The PCR products were analizing by capillar electroforesis using the Beckman Coulter CEQ2000 automatic DNA sequencer.

Taq I primer

*Eco*RI primer	CCT	AAC	AAG	CAT	ACG	CAG
AAC	2	_	_	8	9	11
AAG	_	_	3	0	3	6
AGC	4	3	1	9	4	2
AGT	1	7	4	1	7	5

Fig. 1. Number of variable bands for various selective primer combinations for AFLP analysis. The 14 most polymorphic combinations (in red) were scored in the 50 haploid progeny

Microsatellite Analysis: gynogenetic embryos were genotiping with 20 loci microsatellite for wich the female resulted heterozigous. Fragment analysis were performance by gel electroforesis in Alf Express of Amershan Pharmacia.

Segregation and Linkage Analysis: Markers that deviated significantly from Mendelians ratios were re-checked and removed if unreliable. Segregation of marker loci was analyzed as an F2 backcross population using MAPMAKER/EXP 3.0 Lander *et al* (1987). Initial grouping of markers was carried out using a LOD of 3.0 and a maximum distance of 50.0 cM. In subsequent analysis, LOD was decreased to 2.2. The map distances were estimated using the Kosambi function.

Results

Out of 12 gynogenetic families available one was selected because the high heterozygosity of the mother for a set of 12 microsatellite loci. Fifty gynogenetic haploid embryos from this female were genotyped for 20 microsatelite loci and 93 AFLP markers resulted from 14 most polymorphic primer combinations (Fig. 1). All molecular markers were tested for Mendelian segregation ratios (α=0.05). A total of 88 molecular markers (68 AFLPs and 20 microsatelliteloci) were analyzed for genetic linkage using a LOD of 3.0 and 2.2, subsequently. The genetic map consisted in 15 linkage groups with 56.8% of the markers linked and 43.2% unlinked (Fig.2).

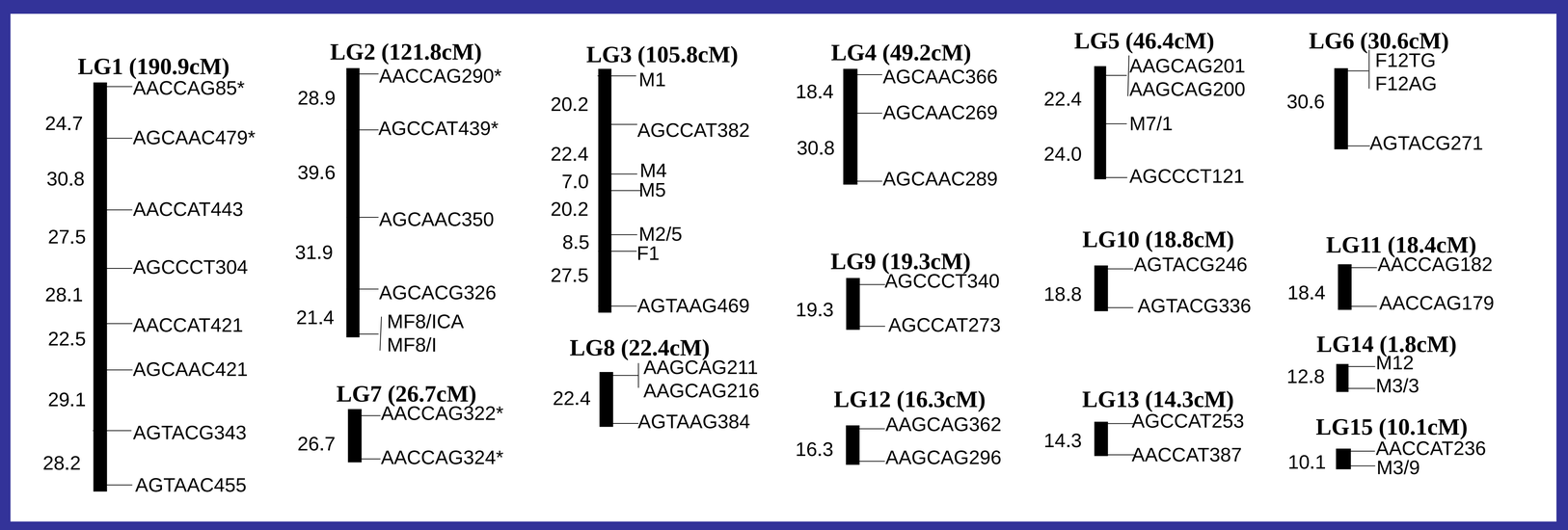


Fig. 1. A linkage map of female turbot, S. maximus. AFLPs are indicated by *Eco* and *Taq* selective nucleotides followed by the fragment size (pb). Linkage groups are numbered by descending size, but are not assigned to individual chromosomes. Markers denoted with "*" are included using α<0,01 from segregation analisis

Discussion

The present map covered 622.9 Kosambi cM. In base of citogenetic data, we can expect a distance about 1000cM for entere turbot genome. It will be necesary increment the analyzed markers to cover the 22 chromosomes of turbot and to test higher LOD scores.

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References:

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