A Microsatellite Linkage Map of the European Sea Bass 

*Dicentrarchus labrax* L.

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ABSTRACT

A genetic linkage map of the European sea bass (*Dicentrarchus labrax*) was constructed from 174 microsatellite markers, including 145 new markers reported in this study. The mapping panel was derived from farmed sea bass from the North Adriatic Sea and consisted of a single family including both parents and 50 full-sib progeny (hierarchical diploids). A total of 162 microsatellites were mapped in 25 linkage groups. Eleven loci represent type I (coding) markers; 2 loci are located within the peptide Y (linkage group 1) and cytochrome P450 aromatase (linkage group 6) genes. The sex-averaged map spans 814.5 cM of the sea bass genome. The female map covers 905.9 cM, whereas the male map covers only 567.4 cM. The constructed map represents the first linkage map of European sea bass, one of the most important aquaculture species in Europe.

The European (or common) sea bass *Dicentrarchus labrax* L. (Moronidae, Perciformes, Teleostei) is an economically significant marine species in European aquaculture. However, as with many cultured marine fish species, the domestication of sea bass, including selection for sex ratio, stress response, resistance to pathogens, and general adaptation to the impact of humans, remains in its infancy.

Genetic management and selection in sea bass would benefit from the development of new genetic tools to assist in reducing inbreeding, lowering losses of farmed fish from infections, improving resistance to suboptimal environmental conditions, and accelerating growth rate. Marker-assisted selection (MAS) can provide a means for increasing response rates to selection, especially for traits such as fat content and shelf life that are difficult to record without killing the target fish (DEKKERS 2004). Species-specific linkage maps composed of highly polymorphic markers represent one of the genetic tools that are essential for conducting comprehensive searches for loci that affect phenotype(s) of interest, particularly those that link to commercially important traits (GARCÍA DE LEÓN et al. 1998; DEKKERS 2004).

Microsatellites, or single sequence repeats (SSRs), represent DNA sequences that show high levels of intraspecific allele polymorphism and are widely distributed within vertebrate genomes (TOOTH et al. 2000). Microsatellite-based linkage maps have been constructed for agriculturally significant avian and mammalian species such as chicken (GROENEN et al. 2000), sheep (MADDOX et al. 2001), cattle (KAPPEL et al. 1997), and pig (ROHRER et al. 1996). Among the economically important fish species, linkage maps based on microsatellite loci have been generated for the Atlantic salmon (GILBEY et al. 2004), Arctic char (WORM et al. 2004), Japanese flounder (COIMBRA et al. 2003), rainbow trout (SATOMOTO et al. 2000), channel catfish (GEFFREY et al. 2001), and Nile tilapia (KOCHER et al. 1998). Here we present a first-generation microsatellite linkage map for the European sea bass that will provide a useful tool for selective breeding and mapping of economically important features in farmed sea bass populations. It will also benefit the evaluation of adaptive traits in natural populations (FARRALL 2004).

MATERIALS AND METHODS

DNA analysis: Two SSR-enriched genomic libraries were generated. Target sequences were enriched prior to cloning using selective hybridization with a 5′ biotinylated (AC)12 oligonucleotide (first library; TSIGENOPOLOUS et al. 2003) and a mix-
Figure 1.—Sex-averaged linkage map in Kosambi centimorgans of the European sea bass consisting of 25 linkage groups.

The cDNA library was constructed from a mixture of sea bass larval stages (details available upon request). A cDNA library from sea bass brain enriched with pituitary gland was kindly provided by A. Canario (Centro de Ciencias do Mar, Faro, Portugal). Nucleotide sequences of tandem-repeat-containing clones from microsatellite-enriched libraries, cDNA clones, and available sequences for genes and mRNA of D. labrax from the GenBank sequence database were used as a source for deriving putative microsatellites with the Tandem Repeat Finder v3.2.1 software (Benson 1999). Flanking primers were designed using the PRIMER3.0 program (Rozen and Skaletsky 1998).

Microsatellites were amplified in a 10-μl volume PCR mixture containing 20 mM Tris-HCl, pH 8.4, 50 mM KCl, 1.0–1.5 mM MgCl₂, 0.2 mM dNTPs, 1 unit of Taq DNA polymerase (Invitrogen Life Technologies, Carlsbad, CA), 50 ng of D. labrax genomic DNA, and 10 pmol of each locus-specific primer. Each forward primer has been labeled with the IRD-700 or IRD-800 fluorescent dye. PCR cycling was carried out on a GeneAmp PCR System 2700 thermocycler (Applied Biosystems, Foster City, CA) with the initial preheating for 9 min following 30 cycles of 30 sec at 96°, 30 sec at 54°–60°, and 60 sec at 72° with a final extension for 7 min at 72°. MgCl₂ concentrations and annealing temperatures (T_a) for each locus are given in supplemental Appendix S1 at http://www.genetics.org/supplemental/. The sequence of new microsatellites was aligned vs. the sequence of D. labrax microsatellites already available in the nucleotide databases using the BLAST 2 SEQUENCES on-line tool (Tatusova and Madden 1999). For each PCR-amplifiable microsatellite, fluorescence-based genotyping of 21 unrelated sea bass individuals was performed using the Li-Cor 4200 DNA sequencer (Li-COR Biosciences, Lincoln, NE). Gel images were analyzed using GENEIMAGER 4.2 software. Observed (H_o) and expected (H_e) heterozygosities and polymorphism information content (PIC) were computed using the POLYMORPHISM program (Botstein et al. 1980).

Linkage mapping analysis: For linkage studies, the Venezia Flis family, consisting of both parents and 50 full-sib progeny (biparental diploids), was genotyped (Chistiakov et al. 2004). Parents were wild and originated from the northern Adriatic Sea. Detection of linkage and map building made use of the CRI-MAP 3.0 software (Green et al. 1990). The assignment of markers to linkage groups was performed by clustering markers showing pairwise LOD scores of ≥4.0. This value was increased from the commonly used threshold of 3.0 to minimize the risk of false linkage being accepted in the large number of two-way tests being performed (Ott 1991). Where there were three or more markers in a linkage group, comprehensive maps (Keats et al. 1991) were derived by selecting the most likely order from those possible by looking at all possible orders of up to six adjacent loci (using the FLIPS6 option in CRI-MAP). Additional markers were added to the map in their most likely position, with the resulting map order being rechecked by looking at all possible orders of up to six adjacent loci in a window that was slid across the whole linkage group until all linked markers had been added to the map. Maps were built by assuming that the recombination rate was the same in both sexes, but orders were also checked allowing the recombination rate to differ between sexes. In no case was a better order found in this latter situation. Once the most likely order had been derived, sex-average and sex-specific linkage distances were estimated for each linkage group assuming the Kosambi (1944) mapping function.

RESULTS

Isolation of microsatellites: We already described the development and characterization of 39 polymorphic SSR markers (DLA0001–DLA0020, DLA0100–DLA0119;
Figure 2.—Male linkage map in Kosambi centimorgans of the European sea bass consisting of 25 linkage groups.

Tsigenopoulos et al. (2003; Chistiakov et al. 2004). In this study, we report 134 additional polymorphic microsatellites, 18 of which (DLA0021–DLA0037, DLA0023K) were selected from the first library, and the remaining 116 loci (DLA0120–DLA0135, DLA0137–DLA0150, DLA0153–DLA0221, DLA0232, DLA0235, DLA0236, and DLA0239–DLA0249) were obtained from the second library (see Appendix S1 at http://www.genetics.org/supplemental/).

In total, 1830 recombinant clones were obtained in both libraries. Of them, 1162 clones contained SSR motifs. Primer pairs were designed for 470 clones and, of these, 277 amplified a PCR product of the expected size. A total of 171 loci showed polymorphism in a population sample of 21 unrelated sea bass individuals. Thus, testing 1830 clones from microsatellite libraries finally yielded 171 (9.3%) polymorphic SSR loci of D. labrax.

We searched for microsatellite nucleotide sequences in 124 nonredundant cDNA clones (109 larval and 15 brain). For 20 SSR-containing cDNA clones, we developed 23 pairs of PCR primers. Testing 17 primer pairs yielded PCR products of the expected size. Finally, 11 of these 17 markers showed polymorphism in the population sample. The polymorphic microsatellites were designated as DLA0223E–DLA0234E (Appendix S1 at http://www.genetics.org/supplemental/). All polymorphic markers were found in anonymous clones, for which no homology with known nucleotide sequences was found.

We also screened for SSR nucleotide sequences of known genes and mRNAs of D. labrax in the GenBank database. We selected eight SSR-containing DNA sequences encoding cytochrome P450 aromatase (CYP19; AJ318516) and recombination activating protein 1 (RAG1; AF137203), somatolactin (AJ277390), β-actin (AJ537421), interleukin 1β (AJ269472), peptide Y (AJ005380), cytochrome P450 aromatase (AY138522), and HMG-CoA reductase (AY424801). Microsatellites located within the RAG1 gene (Venkatesh et al. 1999) and at the 3'-untranslated region of mRNAs encoding aromatase and peptide Y were found to be polymorphic. The polymorphic markers were designated DLA0222RAG1, DLA0237PY, and DLA0238CYP19 (Appendix S1 at http://www.genetics.org/supplemental/). Therefore, a total of 14 type I polymorphic microsatellites were developed from cDNA clones and known genes of sea bass.

Characteristics of polymorphic microsatellites: To date, a total of 200 polymorphic SSRs have been characterized from genomic DNA of D. labrax. These contain 53 markers previously reported by García de León et al. (1995), Castilho and McAndrew (1998), Ciftci et al. (2002), Tsigenopoulos et al. (2003), and Chistiakov et al. (2004) and 146 microsatellites developed in this study. In addition, one locus, SaGT41b, isolated from the gilthead sea bream, Sparus aurata, has been used successfully on genomic DNA of sea bass (Batargias et al. 1999).

For the 200 SSRs, the number of alleles ranged from 2 to 17, with an average of 7.7 alleles per marker (Appen-
Figure 3.—Female linkage map in Kosambi centimorgans of the European sea bass consisting of 25 linkage groups. Twenty-one linkage groups comprised three and more loci whereas the remaining 4 groups each contained two SSRs.

Two of the 11 type I markers mapped, DLA0329PY and DLA0239CYP19, are located within genes encoding peptide Y (Cerda-Reverter et al. 1998) and cytochrome aromatase P450 (Dalla Valle et al. 2002), respectively. They therefore represent the first two mapped genes of the European sea bass that are situated in two separate linkage groups, LG1 (peptide Y) and LG6 (cytochrome aromatase P450) (Figure 1).

The current sex-averaged map spans 814.5 cM of the European sea bass genome. In the map, the intermarker distance ranged from 0 to 32.1 cM, with an average of 5.03 cM. Sixty percent of the intervals between markers varied from 0 to 5 cM, 19% ranged from 5 to 10 cM, 15% varied from 10 to 20 cM, and 6% were >20 cM. The map contains 138 genetically separated markers. A total of 12 microsatellites remained unlinked.

Sex-specific maps have also been constructed. The length of the male map is 567.4 cM, with an average intermarker distance of 3.5 cM (Figure 2) whereas the female map is 905.9 cM long, with an average intermarker spacing of 5.59 cM (Figure 3). The male map comprises linkage groups ranging in length from 0 to 128.7 cM (Figure 2) while the female map contains linkage groups ranging in length from 0 to 178.7 cM.

dixS1 at http://www.genetics.org/supplemental/). The observed heterozygosity ranged from 0.048 to 1.0 (average of 0.718) and the PIC from 0.048 to 0.923 (average of 0.676). A subset of 112 (56%) of 200 microsatellites is highly informative, with an expected heterozygosity of 0.75 and higher and with a PIC value of 0.7 and higher. The 200 SSRs are unique sequences, which have been aligned against each other with no significant similarities being found.

Most of these microsatellites consist of dinucleotide tandem repeats. However, there are also 36 tetra-, 4 hexa-, 4 tri-, and 2 pentanucleotide loci. One marker, DLA0198, actually represents a variable number of tandem repeats because it contains repeat units with an 11-bp consensus sequence.

**Linkage mapping:** The map reference family was polymorphic for 174 microsatellite loci including 11 type I markers and 163 type II SSRs. A null allele was detected in the genotype of the male parent for the loci DLA0023K, DLA0026, and DLA0249. For three markers (DLA0125, DLA0198, and DLA0205), a null allele was identified in the genotype of the female parent. We carefully took these cases into consideration to avoid mapping errors.

The 162 mapped markers were assembled into 25 linkage groups, with an average of six SSRs per linkage group, ranging in length from 1.0 to 147.2 cM (Figure 1).
Thus, the female-to-male (F:M) recombination rate with coding markers will be a prerequisite for these pairs of 0.124 in females and 0.084 in males. Rates (in both sexes with an average recombination fraction of monosex cultures, and acceleration of male growth estimated. A total of 248 pairs of loci were informative for the feminization of farmed populations, breeding informative (i.e., heterozygous) in both parents were estimated. A total of 248 pairs of loci were informative in both sexes with an average recombination fraction in these pairs of 0.124 in females and 0.084 in males. Thus, the female-to-male (F:M) recombination rate ratio in these pairs was 1.48:1.

DISCUSSION

We have already reported on the linkage relationships among 39 microsatellite markers of sea bass, which resulted in the construction of a preliminary linkage map comprising 23 loci clustered in eight linkage groups with a total length of 87.8 cM (Tsigenopoulos et al., 2003; Chistiakov et al., 2004). Here we present a first-generation sex-averaged linkage map of D. labrax containing 25 linkage groups and covering 814.2 cM. In the haploid set of D. labrax, cytological observations identified 24 chromosomes (Figure 4; Sola et al., 1993). Using the method of Postlethwait et al. (1994), we estimated the map size of sea bass at 1709.5 cM. This estimate is similar to the value of tilapia (Kocher et al., 1998), medaka (Naruse et al., 2000), and Japanese flounder (Coimbra et al., 2003).

This first-generation linkage map will undoubtedly evolve as more markers are added, with some pairs of linkage groups coalescing into a single linkage group and single linkage groups possibly splitting. We expect that the addition of new markers should ultimately condense the current map into 24 linkage groups corresponding to the number of chromosome pairs in European sea bass.

The recombination rate has been known to differ between sexes in numerous vertebrates, with a higher prevalence of recombination in females compared to males. F:M recombination rate ratios lie between 1.0 and 2.0 in higher vertebrates [e.g., human (Dib et al., 1996), mouse (Dietrich et al., 1996), dog (Mellersh et al., 1997), and pig (Mikawa et al., 1999)]. In lower vertebrates, the F:M ratio is known to exceed 2.0; in zebrafish, rainbow trout, and Atlantic salmon, the F:M ratio was detected to be 2.74:1, 3.25:1, and 8.26:1, respectively (Sakamoto et al., 2000; Singer et al., 2002; Coimbra et al., 2003; Moen et al., 2005). In Japanese flounder, the recombination rate was unusually high in males (7.4 times) compared to females (Coimbra et al., 2003). In sea bass, the calculated map length is 1.6-fold higher in females than in males. Therefore it represents a value typical for most vertebrate species, for which sex recombination rate has been measured. However, in sea bass, this ratio is the lowest among those reported among marine fishes.

The current linkage map is sufficiently dense to be successfully applied to the mapping of QTL. In addition, both mapped genes (Cerda-Reverter et al., 1998; Dalla Valle et al., 2002) might be involved in economically significant traits such as sex determination, response to stress, and growth rate. The future identification of sex-determining loci will support the use of MAS in sea bass for the feminization of farmed populations, breeding of monosex cultures, and acceleration of male growth rates (Dekkers, 2004). Further enrichment of the linkage map with coding markers will be a prerequisite for comparative mapping with those bony fish species for which a high-density map has been available (Naruse et al., 2000; Woods et al., 2000).

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LITERATURE CITED


