DOI: 10.1534/genetics.107.075416

A Microsatellite Genetic Map of the Turbot (*Scophthalmus maximus*)

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Manuscript received May 5, 2007
Accepted for publication October 2, 2007

ABSTRACT

A consensus microsatellite-based linkage map of the turbot (*Scophthalmus maximus*) was constructed from two unrelated families. The mapping panel was derived from a gynogenetic family of 96 haploid embryos and a biparental diploid family of 85 full-sib progeny with known linkage phase. A total of 242 microsatellites were mapped in 26 linkage groups, six markers remaining unlinked. The consensus map length was 1343.2 cM, with an average distance between markers of 6.5 ± 0.5 cM. Similar length of female and male maps was evidenced. However, the mean recombination at common intervals throughout the genome revealed significant differences between sexes, ~1.6 times higher in the female than in the male. The comparison of turbot microsatellite flanking sequences against the *Tetraodon nigroviridis* genome revealed 55 significant matches, with a mean length of 102 bp and high sequence similarity (81–100%). The comparative mapping revealed significant syntenic regions among fish species. This study represents the first linkage map in the turbot, one of the most important flatfish in European aquaculture. This map will be suitable for QTL identification of productive traits in this species and for further evolutionary studies in fish and vertebrate species.

THE turbot (*Scophthalmus maximus*; Scophthalmidae; Pleuronectiformes) is a flatfish of great commercial value, which has been intensively cultured during the last decade. The decay of turbot fisheries has been accompanied by an increase of its domestic production by up to 7120 tons in 2006 (80% from Spain; Federation of European Aquaculture Producers). Therefore, it represents one of the most promising marine species in European aquaculture. The genetic improvement of turbot will be necessary to achieve large-scale aquaculture success in a highly competitive world market. The control of inbreeding, avoiding the loss of genetic diversity, the identification of sex determining mechanisms for manipulating sex ratios, and the selection of broodstock for disease resistance and growth rate are currently the main goals for improving turbot production. Microsatellite markers have been applied in this species to evaluate wild and cultured genetic resources (Bouza *et al.* 2002) and for parentage analyses, as a support in selection breeding programs (Castro *et al.* 2003, 2004). Recently, the development of genetic markers in turbot has greatly increased, particularly regarding microsatellites (Pardo *et al.* 2006, 2007). Compared to other vertebrate and many fish species, the turbot genome is small (Cuñado *et al.* 2001; Hardei and Hubert 2004), about four times smaller than the human genome, and one of the smallest genomes among farmed fish (Wang *et al.* 2007). At present, the knowledge of turbot genome organization is limited to the well-established mitotic and meiotic karyotypes (Bouza *et al.* 1994; Cuñado *et al.* 2001, 2002; Pardo *et al.* 2001), which revealed an $n = 22$ haploid chromosome number and no sex-linked chromosome heteromorphisms. No linkage groups or conventional genetic maps based on molecular markers have been reported to date in this species.

Genetic maps constitute essential organizational tools for genomic research (Sewell *et al.* 1999). Among the most important applications of genetic maps for aquatic organisms are the mapping of monogenic traits and the identification of quantitative trait loci (QTL) for complex traits of productive interest, which can be applied to marker-assisted selection (Danzmann and Gharbi

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2001; Yu and Guo 2006). This would also lead to the eventual characterization of relevant genes through positional cloning or candidate gene strategies (Danzmann and Gharbi 2001; Chistiakov *et al.* 2005; Colosimo *et al.* 2005). Additionally, genetic maps provide a suitable support for the knowledge of genome organization and evolutionary studies through comparative mapping (Shimoda *et al.* 1999; Naruse *et al.* 2000; Jaillon *et al.* 2004; Kai *et al.* 2005; Cristescu *et al.* 2006; Wang *et al.* 2007). Different markers can be applied for constructing genetic maps (Solignac *et al.* 2004). Microsatellites probably constitute the best choice for medium-high density maps, because they are highly polymorphic, codominant, widely distributed throughout the genomes, and readily assayable using polymerase chain reaction (Shimoda *et al.* 1999; Kai *et al.* 2005; Wang *et al.* 2007).

For most terrestrial animals of scientific or productive interest, pedigrees involving inbred lines and backcrosses have been constructed to simplify mapping procedures (Sewell *et al.* 1999). These approaches have hardly been applied in nonmodel fish species, because available pedigrees are mainly restricted to a few highly specialized farms involved in genetic selection programs. To counterbalance these limitations, chromosomal manipulation techniques represent a powerful strategy for linkage analysis in fish (Lie *et al.* 1994; Kocher *et al.* 1998; Naruse *et al.* 2000). Among them, haploid gynogenetic and androgenetic progenies retaining exclusive maternal and paternal genomes, respectively, offer advantages in assisting linkage mapping, especially with dominant markers such as RAPDs and AFLPs. Additionally, the diploid gynogenetics constitute a powerful tool for half-tetrad analysis, aimed at locating centromeres and establishing an association between markers and linkage groups through joint-segregation analysis (Danzmann and Gharbi 2001; Zimmerman *et al.* 2004).

For any given species, the information contained within different maps can be further enhanced when they are synthesized into a single consensus map. Mapping with multiple populations provides relevant advantages, since a larger number of loci can be placed onto a single map providing larger genomic coverage. These multipoint mapping studies have assisted in the assignment of linkage groups to chromosomes, have provided evidence for chromosomal rearrangements, and they represent the basis for comparative studies among related species (Sewell *et al.* 1999). Consensus maps have been constructed in different animal and plant species (Sewell *et al.* 1999), but they are relatively scarce in nonmodel fish to date (Sakamoto *et al.* 2000; Lee *et al.* 2005; Gharbi *et al.* 2006).

The aim of this study was to construct a first-generation genetic map in turbot. One of the primary goals of our linkage analysis was to map as many genetic markers as possible to obtain a general order on a single reference map of the species. Furthermore, we were concerned about the integration of linkage data from different mapping populations into a single consensus map applicable for assisting the mapping of monogenic traits and QTL identification in the turbot. DNA preservation of the haploid and diploid reference families will enable us to progressively incorporate new molecular markers into the turbot genetic map, including tags within expressed sequences and other dominant or codominant markers used for genomic screening and assisted selection strategies (RAPDs, AFLPs, and microsatellites).

MATERIALS AND METHODS

Mapping populations: Two families were selected for linkage analysis in this study: a haploid gynogenetic progeny from a single diploid turbot female (haploid family, HF) and a diploid biparental pedigree from two genetically heterogeneous parents (diploid family, DF).

HF: Six haploid gynogenetic progenies were obtained at the facilities of the Instituto Español de Oceanografía (Vigo, Spain) in 2002 following the procedure by Piferrer *et al.* (2004). Six diploid females and their respective donor-sperm males, all of wild origin, were used for this purpose. Both parents and 20 haploid embryos of each family were genotyped for diagnostic microsatellites to confirm their haploid constitution and the exclusive maternal inheritance, as previously reported by Castro *et al.* (2003). The most informative haploid family for linkage mapping was selected after genotyping the six females for the 30 turbot microsatellite loci available in 2003 (Coughlan *et al.* 1996; Estoup *et al.* 1998; Iyengar *et al.* 2000; Bouza *et al.* 2002). The HF used to construct the turbot linkage map included the most heterozygous female and a total of 96 haploid embryos. The number of offspring was chosen taking into account the statistical power to detect a minimum and a maximum intermarker distance of 5 cM and 35 cM, respectively ($P < 0.05$; Lie *et al.* 1994).

DF: This mapping population was obtained from the genetic breeding program of the company Stolt Sea Farm, highly specialized in turbot production. A three-generation pedigree was obtained by crossing two unrelated and genetically divergent grandparents coming from different natural populations of the Atlantic area. Eighty-five offspring from a cross between two first-generation individuals were used to construct the linkage map.

DNA analysis: Genomic DNA was extracted from the preserved samples (alcohol 96%, 4°) of the female parent within the HF (muscle tissue) and the juveniles, parents, and grandparents within the DF (muscle tissue) using a standard phenol–chloroform protocol. DNA from the haploid embryos of the HF was isolated using a Chelex protocol (Walsh *et al.* 1991).

Microsatellite markers: Two hundred eighty-six microsatellite loci used in this study were isolated at the University of Santiago de Compostela (Sma-USC loci) using eight enriched libraries (Pardo *et al.* 2005, 2006, 2007). These loci had been reported as useful for population and linkage analysis by these authors. Another 30 loci had been previously reported in this species by different authors (Coughlan *et al.* 1996; Estoup *et al.* 1998; Iyengar *et al.* 2000; Bouza *et al.* 2002). Polymerase chain reactions (PCRs) were then carried out to amplify an initial sample of 316 microsatellites. After checking for polymorphism and technical resolution in the mapping populations, the following 248 markers were included for mapping:

(i) 8 loci reported by Estoup *et al.* (1998) and 30 loci by Pardo *et al.* (2006); (ii) 3 loci published by Coughlan *et al.* (1996); (iii) the locus Smax-04b reported by Bouza *et al.* (2002); (iv) 13 loci isolated by Iyengar *et al.* (2000); (v) 4 loci isolated by Pardo *et al.* (2005); and (vi) 189 markers isolated by Pardo *et al.* (2007). Microsatellite PCR amplifications were carried out as previously reported with slight modifications. Genotyping was conducted on an ABI 3100 DNA sequencer and analyzed using the GENEMAPPER, version 3.7 software (Applied Biosystems, Foster City, CA). Information about the panel of loci mapped in this study is summarized in supplemental Table S1 at http://www.genetics.org/supplemental/ (map location, primer sequences, references, GenBank accession codes, PCR conditions, and polymorphism estimates).

Map construction: *Marker genotyping:* The mother of the HF was genotyped for the 248 selected microsatellites to construct an initial linkage map. Sixty-seven loci resulted monomorphic and 181 loci were then analyzed in the progeny. A subset of 81 markers, uniformly distributed across all linkage groups (LGs) in the HF map, was genotyped in the DF for anchoring. These markers together with those monomorphic in the HF were genotyped in the DF, bringing the total number of markers typed in this family to 148.

Data analysis: The haploid and diploid genetic maps were constructed independently. The marker data in the HF represented the population of segregating meiosis from a single female (maternal data set). The DF data were analyzed to develop an averaged-sex linkage map. In addition, the DF genotypes were divided into two data sets to construct independent male and female linkage maps: DF maternal (DFmat) and DF paternal (DFpat), representing the meiotic segregation from each parent (via female and male, respectively).

Linkage analysis in mapping populations: The software JoinMap 3.0 (Van Ooijen and Voorrips 2001) was used for map construction starting from all haploid and diploid mapping populations (HF, DF, DFpat, DFmat). The genotypes of the haploid gynogenetic progeny were coded as JoinMap population type HAP, with linkage phase unknown. The segregation data from each parent of the diploid family (DFpat and DFmat) were also coded in a HAP configuration with known linkage phase. DF data were coded as JoinMap population type CP and analyzed within a known-phase model. Chi-square values were calculated for all individual markers using JoinMap to detect deviation of gametic segregation from the expected Mendelian ratios (1:1 in the haploid population; 1:1, 1:1:1:1, or 1:2:1 in the diploid population; $\alpha = 0.05$). Bonferroni correction was considered for multiple tests.

Clustering of markers was performed using JoinMap 3.0 with a LOD threshold >3.0 for both mapping populations. The order of adjacent triplets of markers was repeatedly tested through an optimized algorithm to ensure marker order. After map construction, the data files were screened for putative double recombinants, which were verified or corrected by reexamining genotypic data. A LOD threshold >3.0 and a recombination threshold <0.40 were established to obtain a framework map from each mapping population. The remaining markers were ordered by setting the LOD threshold to 2.0 and were represented as accessory markers in their most likely position. A few accessory markers that could not be ordered with a log-likelihood support were represented to the right of the nearest framework marker. Once the most likely order was obtained, genetic distances were estimated for each LG applying the Kosambi mapping function (Kosambi 1944). Sex-averaged and sex-specific linkage distances were estimated from segregation data sets of the diploid population (DF, DFpat, DFmat). The graphic maps were generated using MAPCHART 2.1 (Voorrips 2002).

Integration of linkage data: An integrated linkage analysis was performed to construct a consensus map using all segregation data from the two mapping populations (HF and DF) using JoinMap 3.0. The average recombination frequencies and combined LOD scores were applied to locate loci in the consensus map, which were mapped in more than one population. The consensus framework map was constructed using the same threshold values of individual maps (LOD >3.0; recombination <0.40). Ordering of markers within LGs was done by setting the LOD threshold to 2.0 as recommended by Stam and Van Ooijen (1995). Only in a few cases, less restrictive parameters were chosen, as reported in other species (LOD <2.0 and recombination <0.499; Seefelder *et al.* 2000). Markers that could not be ordered with a log-likelihood support were represented as accessory markers in their most likely position with respect to the nearest framework marker. The graphic maps were generated using MAPCHART 2.1 (Voorrips 2002).

Comparison of meiotic recombination rate among parents: Common marker pairs were chosen for comparing the same genomic intervals across the different maps. A minimum of 40 offspring were used in all pairwise comparisons. Nonparametric rank-order tests were applied for global recombination frequency differences between maternal and paternal data sets in the DF and between females of the HF and the DF.

Sequence comparison: Individual sequences of turbot genomic clones containing the microsatellite loci mapped in this study were compared by NCBI-BLAST under default settings against the downloaded genomic sequences of model fish: *Tetraodon nigroviridis*, *Takifugu rubripes*, and *Danio rerio*. Hits with $e < 10^{-5}$ were considered as significant (Stemshorn *et al.* 2005). The *T. nigroviridis* genome (ver. 7.0) was downloaded from ftp://ftp.ensembl.org/pub/current_tetraodon_nigroviridis/data/fasta. The *T. rubripes* genome (ver. 4.0) was downloaded from ftp://ftp.ensembl.org/pub/current_takifugu_rubripes/data/fasta. Genomic sequences of *D. rerio* were obtained from ftp://ftp.ensembl.org/pub/current_danio_rerio/data/fasta.

RESULTS

Genetic markers and segregation analysis: Starting from 316 microsatellites, a total of 248 genetic markers that segregated in the mapping populations of turbot (HF and DF, respectively) were identified. Null alleles were consistently detected at four loci in the DF (Sma-USC64, 87, 115, and 175) and segregation at these loci was considered only at the heterozygous parent, which did not carry the null allele.

Twenty-three microsatellites (8/181 for the HF, 4.4%; 15/148 for the DF, 10.1%; supplemental Figures S1 and S2 at http://www.genetics.org/supplemental/) exhibited significant segregation distortion ($P < 0.05$) from Mendelian expectations. Only one and four deviations, respectively, were significant after Bonferroni correction in the HF and DF. Distorted loci appeared scattered across different LGs, and only four pairs of loci appeared associated in the same LG (two in each of the reference families). Several other loci in their vicinity did not evidence distorted segregation, precluding an explanation based on deleterious alleles. The higher impact of distorted loci at the DF was due to low technical resolution at five loci. Excluding these, the percentage

TABLE 1

Number of markers and map length for each LG of turbot

Consensus map			Haploid data			Diploid data			Diploid paternal data			Diploid maternal data		
LG	No. markers	Length (cM)	LGH[a]	No. markers	Length (cM)	LGD[a]	No. markers	Length (cM)	LGDp[b]	No. markers	Length (cM)	LGDm[b]	No. markers	Length (cM)
LG1	14	115.1	$LG1H_1$	4	19.5	LG1D	9	77.9	LG1Dp	5	72.1	LG1Dm	6	18.7
			$LG1H_2$	5	11.2									
LG2	21	96.9	LG2H	14	86.2	$LG2D_1$	11	23.3	LG2Dp	5	23.0	$LG2Dm_1$	7	57.1
						$LG2D_2$	2	5.7				$LG2Dm_2$	2	5.7
LG3	11	80.0	LG3H	9	73.9	LG3D	6	44.7	$LG3Dp_1$	4	19.9	$LG3Dm_1$	2	2.6
									$LG3Dp_2$	2	7.6	$LG3Dm_2$	3	2.5
LG4	6	79.9	LG4H	5	25.0	LG4D	2	14.9	LG4Dp	6	31.5	LG4Dm	2	29.3
LG5	13	79.4	LG5H	8	73.5	LG5D	7	39.3	LG5Dp	5	33.9	LG5Dm	5	50.3
LG6	12	78.0	LG6H	8	51.5	LG6D	7	26.5	LG6Dp	3	16.4	$LG6Dm_1$	4	34.3
												$LG6Dm_2$	2	2.7
LG7	12	70.7	LG7H	9	71.3	$LG7D_1$	2	16.9	LG7Dp	3	5.1	LG7Dm	2	7.4
						$LG7D_2$	4	13.3						
LG8	6	66.4	LG8H	3	16.3	LG8D	6	63.7	LG8Dp	3	24.3	$LG8Dm_1$	4	31.5
												$LG8Dm_2$	2	2.7
LG9	9	64.2	LG9H	4	47.7	LG9D	8	45.0	LG9Dp	7	42.8	$LG9Dm_1$	2	13.7
												$LG9Dm_2$	2	10.3
												$LG9Dm_3$	2	6.5
LG10	12	62.4	LG10H	8	57.4	LG10D	7	24.4	$LG10Dp_1$	5	17.0	$LG10Dm_1$	2	5.9
									$LG10Dp_2$	2	6.3	$LG10Dm_2$	3	2.9
LG11	11	62.1	$LG11H_1$	7	57.5	LG11D	6	84.1	LG11Dp	5	63.7	LG11Dm	4	6.4
			$LG11H_2$	3	16.5									
LG12	14	62.1	LG12H	10	43.5	LG12D	6	21.8	LG12Dp	4	19.3	$LG12Dm_1$	2	16.5
												$LG12Dm_2$	2	0.0
LG13	13	60.1	$LG13H_1$	9	45.9	LG13D	6	3.7	LG13Dp	5	26.1	LG13Dm	5	29.1
			$LG13H_2$	2	4.3									
LG14	11	58.0	LG14H	10	66.0	LG14D	3	5.0	LG14Dp	2	6.4	LG14Dm	3	3.7
LG15	11	54.5	LG15H	7	34.8	LG15D	9	39.9	$LG15Dp_1$	4	6.5	LG15Dm	4	17.8
									$LG15Dp_2$	2	5.8			
LG16	11	49.4	LG16H	8	48.4	LG16D	6	37.3	LG16Dp	4	26.8	LG16Dm	6	28.0
LG17	9	49.2	LG17H	7	51.2	LG17D	5	43.4	LG17Dp	3	37.3	LG17Dm	5	38.0
LG18	3	37.3	—	—	—	LG18D	3	37.3	—	—	—	LG18Dm	3	38.5
LG19	9	30.7	LG19H	8	23.0	LG19D	3	8.9	LG19Dp	2	6.2	LG19Dm	2	20.6
LG20	5	24.5	$LG20H_1$	3	24.5	LG20D	2	2.6	LG20Dp	2	2.6	—	—	—
			$LG20H_2$	2	2.3									
LG21	7	18.0	LG21H	4	28.6	LG21D	6	8.7	LG21Dp	4	0.0	LG21Dm	4	16.2
LG22	8	17.8	LG22H	8	16.0	LG22D	2	22.4	LG22Dp	2	27.1	LG22Dm	2	18.0
LG23	5	16.6	LG23H	4	34.3	LG23D	4	12.4	LG23Dp	4	4.1	—	—	—
LG24	3	6.7	LG24H	3	0.0	LG24D	2	12.0	—	—	—	—	—	—

(*continued*)

TABLE 1

(Continued)

LG	Consensus map		Haploid data			Diploid data			Diploid paternal data			Diploid maternal data		
	No. markers	Length (cM)	LGH[a]	No. markers	Length (cM)	LGD[a]	No. markers	Length (cM)	LGDp[b]	No. markers	Length (cM)	LGDm[b]	No. markers	Length (cM)
LG25	4	3.3	—	—	—	LG25D	4	3.3	LG4Dp[c]			LG25Dm	4	5.2
LG26	2	0.0	—	—	—	LG26D	2	0.0	LG10Dp2[c]			LG26Dm	2	0.0
Range	2–21	0–115.1	Range	2-14	0–86.2	Range	2–11	0–84.1	Range	2–7	0–72.1	Range	2-7	0–57.1
Mean	9.3	51.7	Mean	6.4	38.2	Mean	5.0	26.4	Mean	3.7	21.3	Mean	3.2	16.8
Unlinked	6		Unlinked	9		Unlinked	8		Unlinked	28		Unlinked	19	
Bins[d]	229		Bins[d]	163		Bins[d]	139		Bins[d]	97		Bins[d]	83	
Total	248	1343.2	Total	181	1030.2	Total	148	738.3	Total	121	531.7	Total	119	522.1
26 LGs			27 LGs			28 LGs			25 LGs			31 LGs		

Correlated LG codes and lowercase numbers are presented to integrate independent maps into the consensus one. LGs of the consensus map, which appeared split into more than one LG in the other maps, were numbered correlatively from the longest to the shortest one.

[a] LGs in the map from haploid and diploid families, respectively.

[b] LGs obtained from paternal and maternal data sets within the diploid family, respectively.

[c] Some markers located in LGs 4 and 10 in the paternal map (LG4Dp and LG10Dp2) were mapped in different LGs in the consensus one (LG25 and LG26, respectively; supplemental Figures S1 and S2 at http://www.genetics.org/supplemental/).

[d] Unique positions in the map.

of deviations was around that expected by chance (6.1%), only two after Bonferroni correction.

Construction of individual linkage maps: The genetic maps constructed from the four data sets, the HF, the DF, the DFpat and the DFmat diploid data, contained 181, 148, 121, and 119 markers, respectively (Table 1). Among these, 156 (86.2%), 105 (70.9%), 93 (76.8%), and 86 (72.3%), respectively, were mapped at LOD >3.0. The linkage data from both mapping populations (HF and DF) contained 76 common informative markers (63 framework markers). They were used to align homologous LGs among mapping populations (supplemental Figures S1 and S2 at http://www.genetics.org/supplemental/) to integrate them into a single consensus map, which consisted of 26 LGs named LG1–LG26 (Figure 1 and Table 1). Correlated numerical codes were used to represent homologous LGs among the maps (LGH, LGD, LGDp, and LGDm in the maps from HF, DF, DFpat, and DFmat data, respectively).

The map length including all markers from the HF was 1030.2 cM, whereas the sex-averaged map from the DF spanned 738.3 cM (Table 1). Similar characteristics in the number and distribution of markers, as well as in the number and size of LGs, were observed for both HF and DF maps (Table 1), excluding the average distance between markers, which was slightly lower in the HF than in the DF map (7.6 *vs.* 8.9 cM; Table 2).

The male and female maps including all markers, obtained from the DFpat and DFmat data sets within the diploid family, showed similar length (531.7 and 522.1 cM, respectively; Table 1). However, there were differences in the number and length of the LGs between both maps (Table 1; supplemental Figures S1 and S2 at http://www.genetics.org/supplemental/). The male map showed a lower number of LGs, with higher length per LG on average (25 LGs; mean LG length, 21.3 cM) than the female map (31 LGs; mean LG length, 16.8 cM; Table 1). In addition, the average distance between markers was lower in the male map than in the female one (8.3 *vs.* 9.7; Table 2). Some informative markers in both parents of the DF were linked in the male map but remained unlinked in the female map, and vice versa. This could be explained by sex-specific differences in recombination across intervals and LGs (see below).

Orthologous markers among homolog LGs were compared for colinearity of marker order. Colinearity was mostly observed among markers of the same LG (supplemental Figures S1 and S2 at http://www.genetics.org/supplemental/). Some discrepancies were due to markers which were not framework in the consensus map (LG5, LG11). Several closely linked markers scattered across different groups appeared interchanged in different maps and one marker at LG13 was discordant between male and female maps. Some of these regions contained quite frequent double crossovers that made a conclusive ordering of markers difficult.

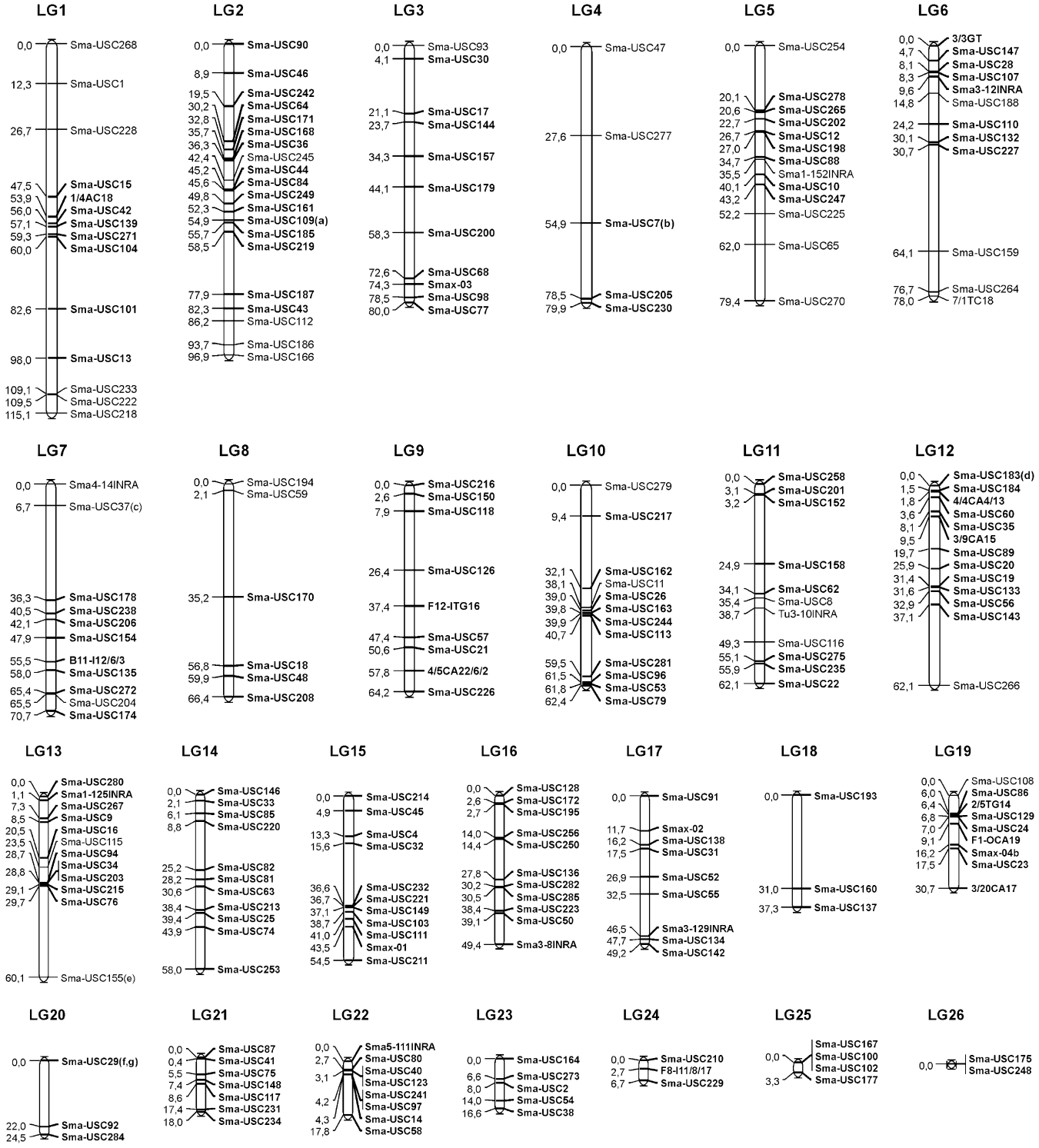

FIGURE 1.—A consensus genetic map for turbot. The integration of the individual maps from the two mapping populations and the four data sets (HF, DF, DFpat, and DFmat) are shown in supplemental Figure 1 at http://www.genetics.org/supplemental/. Framework markers (LOD >3.0) are presented in boldface type. Seven markers that could not be ordered with a log-likelihood support are represented as accessory markers at the right of the nearest linked marker: (a) Sma-USC51 (31.2 cM), (b) B12-IGT14 (14.9 cM), (c) Sma-USC224 (16.9 cM), (d) Sma-USC169 (0.0 cM), (e) Sma-USC27 (4.3 cM), (f) Sma-USC176 (2.6 cM), and (g) Sma-USC95 (4.9 cM).

TABLE 2

Estimated genome length of turbot maps

	Consensus	HF	DF	DFpat	DFmat
Min. gaps[a]	10	14	14	31	28
Max. distance (cM)[b]	33.4	31.5	29.4	29.3	29.3
Total markers	248	181	148	121	119
Intervals	207	135	83	64	54
Resolution (cM)[c]	6.5	7.6	8.9	8.3	9.7
Genome length (cM)	1802.6	1603.2	1266.8	1580.8	1520.9
Framework markers	199	156	105	93	86
Intervals	171	127	75	64	52
Resolution (cM)[c]	5.6	7.1	8.3	8.3	9.4
Genome length (cM)	1354.9	1421.4	1045.7	1502.2	1413.8

Genome length was estimated as in *Danio rerio* by Postlethwait *et al.* (1994) and Shimoda *et al.* (1999).

[a] Minimum number of unfilled gaps in the maps following Postlethwait *et al.* (1994), including the unlinked markers plus the additional LGs relative to the haploid number of chromosomes in turbot (22; Bouza *et al.* 1994).

[b] Maximum intermarker distance in each of the maps.

[c] Average distance between two markers.

The genome length was estimated from each individual turbot map on the basis of both the total number of markers and the subset of framework markers (HF, DF, DFpat, and DFmat; Table 2) as reported in *D. rerio* (Postlethwait *et al.* 1994; Shimoda *et al.* 1999). When only the framework markers were considered, lower estimates were obtained (Table 2), mostly due to the accessory markers that were quite frequently located at the ends of LGs, increasing their length (Figure 1).

Construction of the integrated consensus map: The resulting consensus map (Figure 1) contained 242 microsatellite markers (80% at LOD >3.0) with 229 unique positions (193 framework bins). Only 6 markers remained unlinked (Sma-USC78, 83, 125, 252, 263, and 269). Twenty-six LGs were found, with an average of 9.3 microsatellites per LG, ranging in length from 0 cM (2 markers in LG26) to 115.1 cM (14 loci in LG1) (Table 1). The total length of the map was 1343.2 cM, and the intermarker distance ranged from 0 to 33.4 cM, with an average of 6.5 cM. In concordance with the individual maps, the terminal location of the accessory markers determined an increase in the length of the consensus map. Seven of them could not be mapped with respect to their nearest markers, mainly due to the very large (as Sma-USC109 *vs.* Sma-USC51, 31.2 cM, LG2) or very small (as Sma-USC183 *vs.* Sma-USC169, 0 cM; LG12) distances between them, and also to the lack of informative adjacent loci. When only the framework markers were considered, the map length was 959.26 cM, with an average intermarker distance of 5.6 cM. In this framework map, 61% of intermarker intervals were <5 cM, 20% ranged between 5 and 10 cM, 14% between 10 and 20 cM, and 5% were >20 cM. The marker order in the consensus map was not different from that defined by each individual map, with a few minor exceptions. Some of the markers located in the LGs DP4 and DP10 of the paternal map, were mapped in different LGs in the consensus map (LG25 and LG26, respectively; Table 1; supplemental Figures S1 and S2 at http://www.genetics.org/supplemental/). These results could suggest the coalescence of the two smallest groups in the consensus map with other major LGs toward the expected number of LGs in the turbot ($n = 22$; Bouza *et al.* 1994).

The estimates of genome length of the turbot consensus map based on the whole (242) and framework (199) markers were 1802.6 cM and 1354.9 cM, respectively (Table 2).

Differences in recombination rate between sexes and families: The availability of common microsatellite markers in the different maps allowed for a comparative evaluation of meiotic recombination rate. Only a slight difference in length was observed between the male and the female maps within the diploid pedigree (531.7 and 522.1 cM, respectively; Table 1). Biased estimates might be explained by uneven sampling of informative loci in both parents, but this was not the case (121 in DFpat *vs.* 119 in DFmat; Table 1). However, when only common informative markers were selected (42 loci; 23 intervals; 17 LGs; supplemental Table S2 at http://www.genetics.org/supplemental/), a significantly higher recombination rate was observed in the female map (Figure 2A; 13.3 ± 2.5 *vs.* 8.3 ± 2.0; $P < 0.05$). The proportion of intervals that showed a higher recombination rate in females was higher than in males (60%). Summing up the length of the common intervals for each LG, it rendered a total length of 191.7 cM and 306.1 cM in the male and female maps, respectively. Thus, the recombination rate in the female resulted 1.6 times higher than in the male. The higher number of LGs in the maternal than in the paternal map (31 *vs.* 25) was in agreement with the higher female recombination rate: five LGs in the male map split into more than one group in the female map (Table 1). However, there were exceptions in some LGs, and some intervals showed higher length in the male, for instance LG22 (Table 1; supplemental Figures S1 and S2 at http://www.genetics.org/supplemental/). On the contrary, the higher number of unlinked markers in the male map might point to the heterogeneous recombination rate among chromosomal intervals in male meioses.

The comparison between the two independent female maps obtained from HF and DFmat data sets revealed the expected length differences due to the different number of informative loci managed (Table 1). The comparison of 13 common intervals across 11 linkage groups evidenced similar recombination rates between both maps (Figure 2B; 20.6 ± 4.1 *vs.* 21.0 ± 3.9; $P > 0.05$; supplemental Table S2 at http://www.genetics.org/supplemental/). Moreover, the average estimates of genome length in both maternal maps were very similar (Table 2).

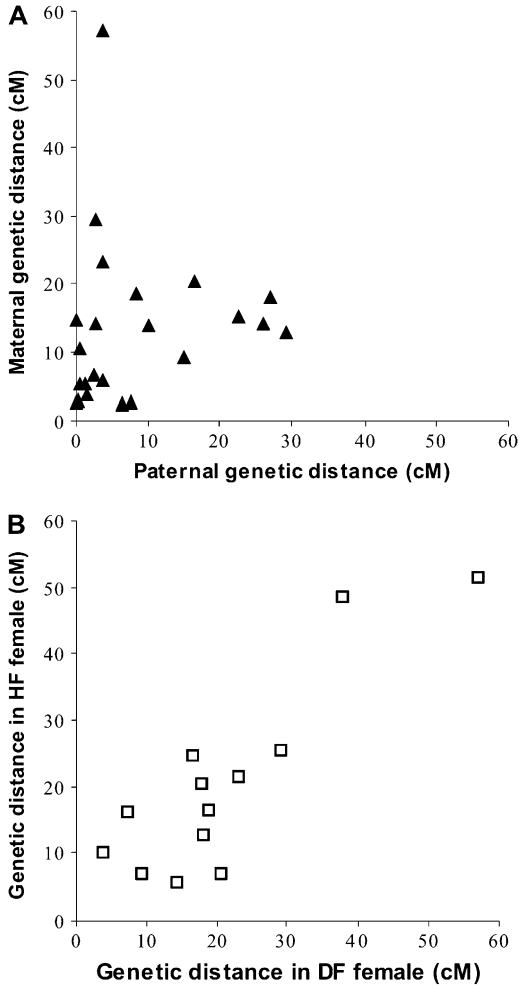

FIGURE 2.—Differences in recombination ratio. (A) Male *vs.* female recombination ratio for pairs of framework markers segregating from both parents of the diploid family (DF). (B) Female recombination ratio for pairs of markers segregating from female parents of both haploid (HF) and diploid (DF) families.

Comparative mapping: BLASTn matches of 219 turbot microsatellite flanking sequences against the *T. nigroviridis*, *T. rubripes* and *D. rerio* genomes were obtained using a significance threshold of $e < 10^{-5}$, most of which were even retained at a significance threshold of $e < 10^{-10}$. Fifty-five turbot sequences (24%) showed high similarity to known genomic DNA sequences of *T. nigroviridis*. Many of these sequences showed a significant match also against the *T. rubripes* genome (43/55; 78%). By contrast, the comparison with *D. rerio* yielded a lower number of significant hits (19; 9%), many of them (38%) matching to two or more different *D. rerio* genomic regions. A lower number of turbot sequences showed multiple matching against Tetraodon (9.1%) and Takifugu (9.4%) genomes.

The distribution of turbot sequences with the ordered map available for the Tetraodon genome, which covers ~64% of the genome sequence (JAILLON *et al.* 2004), allowed assessing large-scale synteny patterns between both species (Figure 3). Eight turbot LGs could not be associated with Tetraodon LGs, in most cases due to lack of significant hits (LGs 4, 9, 14, 20, 24, and 26). In the two remaining ones (LG8 and LG11), hits were only found on genomic fragments that were not yet anchored to the Tetraodon genetic map. Seven turbot LGs (6, 15, 18, 19, 21, 23, and 25) mapped each to a single Tetraodon chromosome (Figure 3). Five turbot groups yielded hits with more than one Tetraodon chromosome (up to 3 for LG13). Despite the limited number of anchoring points, the comparison revealed several syntenic regions, some of them including three markers. For instance, three loci on turbot LG16 (Sma-USC136, Sma-USC285, and Sma-USC223) spanning 10.6 cM defined a syntenic block on Tetraodon chromosome 19. Similarly, turbot LG5 contained three markers with a total map length of 14 cM (Sma-USC265, Sma-USC12, and Sma-USC88) showing putative homology with chromosome 1 in the Tetraodon genome. Two completely linked markers in turbot LG25 (Sma-USC167 and Sma-USC102) defined a microsyntenic block on LG5 of the Tetraodon genome. The significant matches against Tetraodon were usually due to highly conserved sequences, with a length of 29–327 bp (average 102 bp) and sequence similarities between 81 and 100%. A few loci with matching flanking sequences showed a conservation of the proper microsatellite in Tetraodon, as has been reported in other comparative mapping studies in fishes (STEMSHORN *et al.* 2005). Even more, 16 of the significant hits with the Takifugu and Tetraodon genomes (37.2%) showed significant homologies using BLASTn ($e < 10^{-5}$) with different gene sequences in several species.

DISCUSSION

This study represents the first genetic map in turbot, a teleost fish of great relevance for fisheries and aquaculture. The consensus linkage map obtained consolidated LGs from different mapping populations, including a known-phase diploid pedigree, related to QTL experiments in turbot, and a haploid family, suitable for future linkage mapping of dominant markers in this species. Furthermore, the integrated map represents an excellent resource from which markers may be selected for future mapping projects within turbot and for comparative studies among fish.

The current consolidated map of 242 microsatellites spans a total length of 1342.2 cM, with 26 LGs. This number exceeded the number of haploid chromosomes of turbot (22; BOUZA *et al.* 1994) and six loci remained unlinked to any other marker; thus at least 10 gaps should be filled to consolidate the turbot map. The discrepancy between the number of LGs and the haploid number of chromosomes has been commonly reported when constructing linkage maps in fish, including that of tilapia, one of the most extensive genetic maps available with a high number of microsatellites

Scophthalmus maximus linkage groups

Tetraodon nigroviridis linkage groups	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	UL
1					3		1																				
2		1	1										1								1						
3													1														
4																											
5	1																								2		
6																	1										
7													1					1									1
8		3																									
9										3																	
10			3												1												
11																											
12												3										1					
13						3																					
14																							2				
15	1																										
16																											1
17																			1								
18							2																				
19																3											
20																											
21																											
UN	1		1		1		2	1		1	1	1				1	1					1					

FIGURE 3.—Syntenies between turbot and *T. nigroviridis* linkage maps. The linkage groups of turbot and Tetraodon were arrayed as columns and rows, respectively. UL, unlinked markers in turbot consensus map. UN, unknown: genome sequences that have not been mapped in Tetraodon. Values in boxes indicate the number of syntenic turbot microsatellite flanking sequences.

(525; LEE *et al.* 2005). It is expected that, with the addition of more markers, some LGs will merge into larger ones, and their number should condense toward the haploid karyotype of the species.

Assuming that markers were randomly distributed, ~99% of the loci are estimated to be located within 15 cM of a marker on the turbot map (JACOB *et al.* 1991; POSTLETHWAIT *et al.* 1994). The fact that only 6 of the 248 markers studied remained unlinked to any other marker supports this estimate. This degree of completeness supports the utility of the consensus map of turbot as a reference tool for future genetic analysis in this species.

Genome coverage and average resolution map: Using the method of POSTLETHWAIT *et al.* (1994), we estimated the genome length for the consensus map of turbot at ~1800 cM (framework map: ~1350 cM). This size map represents three-quarters of the female map plus one-quarter of the male map lengths, since the HF and DF maps contributed equally to the consensus, and the DF map was averaged between both sexes. The concordance between the two independent female maps (HF and DFmat), in genome length, colinearity of LGs, and recombination rate, suggests an average female genome length of ~1560 cM (framework female maps: ~1400 cM). The female estimate was similar to that obtained from the male parent, 1551.8 cM (framework map: 1502.2 cM), yielding a sex-averaged genome length in turbot of ~1500 cM. This global estimate is similar to the sex-averaged values reported for other fish species using the same method, as in *Dicentrarchus labrax* (CHISTIAKOV *et al.* 2005). However, the turbot represents one of the smallest genomes among cultured fish (WANG *et al.* 2007), with estimates of haploid *C*-values ranging from ~0.65 pg (CUÑADO *et al.* 2001) to 0.86 pg (HARDIE and HEBERT 2004). The estimated genome size in turbot (<800 Mb) would render 530 kbp/cM on average, although the relationship between physical and genetic distance may vary among regions of the turbot genome and between sexes (as seen below). Nevertheless, this figure in turbot was lower than in other fish and very similar to that found in the model fish *T. rubripes* with half-genome size (KAI *et al.* 2005), which suggests a higher recombination rate in turbot than in other fish. This is in accordance with the highest ratio between the synaptonemal complex length and the DNA content observed in this species among bony fish, related in turn to a higher recombinational ratio per physical length (CUÑADO *et al.* 2001).

Family and sex variation in recombination rate: The studies involving different mapping populations in fish are relatively scarce to date. In this study, the comparison between the individual maps in turbot did not reveal any evidence of major intraspecific chromosomal rearrangements among the three parents involved. This is in agreement with previous karyotypic data, which revealed a very stable karyotype both in chromosome and arm number in this species (BOUZA *et al.* 1994; PARDO *et al.* 2001). The interindividual differences in genetic maps reported in some fish species, such as salmonids, have been related to karyotypic polymorphisms within species (SAKAMOTO *et al.* 2000).

The similarity in the overall map length between sexes hides the fact that the distribution of recombination across chromosomes can be very different between males and females. Biased estimates might be expected by unequal sampling of loci in the biparental family. However, evidence for a higher recombination rate in

the female was suggested when sex-specific recombination was estimated only for common informative loci in both parents. Recombination rate has been reported to be lower in male than in female meioses in several teleost fish (Sakamoto *et al.* 2000; Waldbieser *et al.* 2001; Wang *et al.* 2007). The female:male recombination rate in this study (1.6:1) is similar to the ratios observed in microsatellite-based maps in *D. labrax* (1.5:1; Chistiakov *et al.* 2005) and *Sparus aurata* (1.2:1; Franch *et al.* 2006), but quite different from the AFLP-based map in other flatfish species [1:7.4, *Paralichthys olivaceous* (Coimbra *et al.* 2003)]. In other fish species, such as Xiphophorus spp. and Oreochromis spp., no significant differences were found between the overall lengths of male and female maps, although sex-specific recombination rates appeared to vary among and within LGs. A similar situation was observed in the turbot in this study. The analysis of sex-specific differences among map intervals has also been reported in other fish species, such as *Oncorhynchus mykiss*, *T. rubripes*, and *Lates calcarifer* (Sakamoto *et al.* 2000; Kai *et al.* 2005; Wang *et al.* 2007).

The averaged 5.6 ± 0.5 cM marker distance of the consensus framework map (~8.3 cM in both sexes: female-averaged and male framework maps) offers enough marker density for genetic dissection of quantitative traits (QTL). For QTL analysis, an intermarker distance <20 cM is generally required (Dekkers and Hospital 2002; Dekkers 2004). In addition, the information on sex-variation in recombination rate across genome regions has practical implications facilitating an efficient experimental design in the genome-wide linkage analysis in turbot. The heterogeneity in recombination rate at common intervals between the mapping families, which reduces the precision of the consensus map, should also be taken into account to accurately interpret the consolidated genetic distances. A decreased average rate of recombination in males would be an advantage for mapping genetic traits in initial low resolution analysis, especially when analyzing QTL (Glazier *et al.* 2002). On the other hand, it would be necessary to use a higher frequency of recombination in females or in males at particular intervals for fine mapping of loci of interest (Kai *et al.* 2005).

Comparative mapping: The comparative mapping of the unique turbot sequences against model fish genomes was in agreement with phylogenetic data, since turbot is more closely related to Takifugu and Tetraodon (Acantopterygii) than to *D. rerio*, within Ostariophysi (Miya *et al.* 2003). Very similar results were obtained using different BLAST thresholds (10^{-5}–10^{-10}) for other Acantopterygii against Tetraodon genome sequences [11%, *Oreochromis niloticus* (Lee *et al.* 2005); 45%, *Cottus gobio* (Stemshorn *et al.* 2005); 30%, *S. aurata* (Franch *et al.* 2006); 23%, *L. calcarifer* (Wang *et al.* 2007)]. The observed syntenic relationships, together with the sequence similarities between the turbot and Tetraodon sequences, suggest true homology of the associated regions. While it is interesting to speculate about the functional role of these sequences (Gaffney and Keightley 2004; Franch *et al.* 2006), they also provide highly useful tools for linking genome information between species. Synteny among species or genera may bring the opportunity to complement initial QTL experiments with candidate gene approaches from homologous chromosomal locations identified in related model organisms (Erickson *et al.* 2004). Nevertheless, caution should be taken due to the occurrence of intrachromosomal rearrangements within the evolutionary history of fish that do not allow direct transfer of all positional information among genomes. Even so, it is possible to trace microsyntenic relationships, even if the whole chromosome segment is rearranged at intra or interchromosomal levels (Stemshorn *et al.* 2005). More detailed comparison of the turbot's unique sequences with the genetic maps of model fish will allow the assessment of large-scale synteny and order patterns among species.

In summary, this study represents the first-generation linkage map in turbot, integrating different mapping population data within a single fish species and linking, by comparative mapping, to other model fish. The genetic map will be applied to identify QTL and genomic regions related to characters of productive and evolutionary interest (disease resistance, growth rate, sex determination) in the turbot genome. The turbot consensus map will also serve as a reference map for genomic analysis in turbot and for comparative genomics in fish.

We are indebted to Lucía Insua, María Portela, Susana Sánchez, María López, and Sonia Gómez for technical assistance. This study was supported by a project from the Xunta de Galicia local government (PGIDIT04RMA261014PR).

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Communicating editor: Y.-X. Fu