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Compilation of mapping resources in turbot (*Scophthalmus maximus*): A new integrated consensus genetic map

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ABSTRACT

The turbot (*Scophthalmus maximus*) is a species of great relevance to European aquaculture and hence might benefit from enhanced genomic resources. The aim of this study was to integrate all previous turbot mapping resources to obtain a dense consensus genetic map. A total of 514 single nucleotide polymorphisms (SNP) and microsatellite markers from reported turbot genetic maps, were used for map construction. Among these markers, 487 (LOD score > 3), including 39% expressed sequence tag (EST)-linked and 61% anonymous, were integrated in 24 linkage groups. The linkage map comprised a total length of 1414 cM (1274.4 cM framework) with 3.3 cM (3.6 cM framework) inter-marker distance, representing an estimated genomic coverage of ~90%. This map was used to refine quantitative trait loci (QTL) screening for sex, because of the relevance of this trait for the turbot industry, which confirmed not only the main sex-determining region at linkage group (LG) 5, but also the involvement of additional significant genetic factors at other linkage groups. Comparative mapping supported the macrosyntenic pattern previously observed when comparing turbot and model fish genomes and enabled identification of candidate genes related to sexual development and reproduction at LG5, such as *DNAJC19* and *Sox2*. The available number of markers in the turbot map could be easily increased to 592 because of the established correspondence between linkage groups of the two main genetic maps, thus approaching 1 marker/Mb based on the turbot genome size. This map represents a useful tool for evolutionary studies and for supporting ongoing marker-assisted selection programs in this species.

1. Introduction

The turbot (*Scophthalmus maximus*) is a flatfish of great commercial value in Europe, and its production has increased very rapidly in recent years, currently numbering ~15,000 tons per year. Turbot is also becoming very popular in other countries around the world, especially in the People's Republic of China, where its production reached ~50,000 tons in 2006 (Ruan et al., 2010). Increasing growth rate, controlling the sex ratio and enhancing disease resistance currently constitute the main goals of genetic breeding programs for this species.

Genetic maps are essential tools to identify genomic regions related to productive characters (Mackay, 2001). This information can eventually be used to identify genes related to productive traits through fine mapping, positional cloning or comparative genomics (Martínez et al., 2009; Moen et al., 2009; Sarropoulou et al., 2008; Wang et al., 2011). Genetic maps are also required for QTL identification, and information on genetic markers associated with QTL can be used in marker-assisted selection (MAS) programs. Although there are few QTL screening studies in commercial fish, QTL for growth and disease resistance have been described in several species (Fuji et al., 2006; Rodríguez-Ramilo et al., 2011, 2013; Sánchez-Molano et al., 2011; Song et al., 2012; Wang et al., 2011).

Genetic maps have been constructed for all model fish species and for the most commonly farmed fish, providing relevant information to understand genome organization and evolution, as well as the genetic basis of complex productive traits (Canario et al., 2008; Danzmann and Gharbi, 2007). This effort has continued recently and increasingly dense EST-enriched genetic maps have been reported in several aquaculture species (Kucuktas et al., 2009; Wang et al., 2011; Xia et al., 2010). Several genetic linkage maps have been published in economically important flatfish species, such as the Japanese flounder (*Paralichthys olivaceus*, Castaño-Sánchez et al., 2010; Coimbra et al., 2003; Kang et al., 2008), Atlantic halibut (*Hippoglossus hippoglossus*, Reid et al., 2007), and half-smooth tongue sole (*Cynoglossus semilaevis*, Liao et al., 2009; Song et al., 2012). The enormous advances in SNP identification and genotyping, mainly due to the decreasing cost of DNA sequencing are facilitating the construction of highly dense genetic maps with thousands of markers (Kai et al., 2011; Lien et al., 2011). Recently, Next Generation Sequencing (NGS) technologies have shown their potential to identify thousands of SNPs (Davey et al., 2011; Wang et al., 2012), thus facilitating the subsequent construction of genetic maps

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(Amores et al., 2011; Houston et al., 2012; Liu et al., 2012). Additionally, another efficient approach to improve mapping is to integrate existing mapping resources to achieve dense genetic maps, as reported in some fish species (Guyon et al., 2010; Kikuchi and Kai, 2012; Nichols et al., 2003; Palti et al., 2011; Song et al., 2012).

The first turbot genetic map (242 anonymous microsatellites; 26 linkage groups (LG)) was reported by Bouza et al. (2007). This map was subsequently enriched with 180 EST-linked markers demonstrating a higher performance for comparative mapping strategies (Bouza et al., 2008, 2012). These maps were previously the main tool to identify QTL and look for candidate genes associated with sex (Martínez et al., 2009), growth (Sánchez-Molano et al., 2011) and resistance to pathogens (Rodríguez-Ramilo et al., 2011, 2013). Recently, another turbot genetic map based on 158 new anonymous microsatellite markers was reported by Ruan et al. (2010).

The aim of the present study was to gather all existing turbot mapping resources to construct an integrated dense consensus map, which would be useful for evolutionary genomic studies and genetic breeding strategies in this species. To achieve this, a group of selected genetic markers from all linkage groups from the Ruan et al. (2010) map was genotyped according to the reference families used by Bouza et al. (2012) to anchor the linkage groups from both maps, thus integrating them in a single map. In addition, candidate genes related to immunity and gonad differentiation previously mapped by Rodríguez-Ramilo et al. (2011) and Viñas et al. (2012), respectively, were included. The higher information content of this new map enables finer detail comparative mapping studies with model fish genomes to identify candidate genes at relevant QTL regions.

2. Materials and methods

2.1. Mapping families

The previously reported mapping families, the haploid (HF) and the diploid (DF) families (Bouza et al., 2007), and the seven additional mapping families used for QTL screening (QF1-QF7; Bouza et al., 2012) were used to genotype new markers and integrate all information into a single consensus map. The HF was obtained from the Instituto Español de Oceanografía (Vigo, Spain) in 2002. Six haploid gynogenetic progeny were obtained from six diploid females and their respective donor-sperm males, all of wild origin. The most informative family was selected after genotyping the mothers for 30 turbot microsatellite loci. DF and QF families were obtained from commercial genetic breeding programs (Stolt Sea Farm SA and Insuiña SA). A three-generation pedigree was obtained by crossing unrelated and genetically divergent grandparents coming from different wild populations of the Atlantic area. The parents and grandparents of this family panel were genotyped for all selected markers to identify informative families for mapping. DF was the main reference family and thus, when informative, this family was used for mapping. QF families were only used when markers were non-informative in DF.

2.2. Mapping resources

The main source of mapping markers came from the Bouza et al. (2012) map, which integrated all previous data from our group (Bouza et al., 2007, 2008; Martínez et al., 2008). This map included 438 SNP and microsatellite markers, 180 EST-linked and 258 anonymous markers. The other major source of markers came from the Ruan et al. (2010) map, which included 158 anonymous microsatellites. Henceforth, we refer to the main turbot maps as TB (Bouza et al., 2012) and TR (Ruan et al., 2010). Finally, minor gene-associated resources (including SNP, restriction fragment length polymorphism (RFLP), microsatellites and random amplification of polymorphic DNA (RAPD) markers) were also integrated: the *MHC-IIb* gene (Rodríguez-Ramilo et al., 2013) and 13 sex-associated markers (Viñas et al., 2012).

2.3. Genotyping

Genotyping effort was employed to establish the correspondence between linkage groups of the TB and TR maps, because the maps did not share any common markers. As such, we selected the minimum number of markers in the TR map which ensured that all linkage groups could be anchored to the TB map. The linkage groups of the TR map were reported separately for male (21 linkage groups) and female (30 linkage groups). There were 26 female linkage groups that shared common markers with male linkage groups, and four linkage groups with exclusively female map markers. All linkage groups from the male map shared markers with linkage groups in the female map. Therefore, we selected 31 markers to anchor all linkage groups of the TR map to the TB map (Table S1). Furthermore, all TR map markers of the most relevant sex-associated linkage group counterparts (LG5 and LG21; Martínez et al., 2009; Viñas et al., 2012) were genotyped for mapping because of the relevance of sex determination for turbot industry (Table S1). In total, 37 microsatellite markers from Ruan et al. (2010) were genotyped to be integrated into the new map. Primers and PCR conditions for amplification were as previously described in Ruan et al. (2010). Markers with positive amplification were first genotyped in parents and grandparents of the reference families, and then genotyped in the offspring of the most informative family. Genotyping was carried out in an ABI 3730xl DNA sequencer using the GENEMAPPER 4.0 software (Applied Biosystems).

2.4. Mapping

Novel genotype data were coded as JOINMAP type HAP population with linkage phase unknown (HF family), and as type CP population with known-phase (DF family). This genotype data set was added to the original data from Bouza et al. (2012), Rodríguez-Ramilo et al. (2013) and Viñas et al. (2012). Mapping procedures were the same as those from Bouza et al. (2012). Briefly, a consensus genetic map was constructed using JOINMAP 3.0 (van Ooijen and Voorrips, 2001) starting from the nine reference families (HF, DF, QF1–7). A LOD threshold of 3.0 and a recombination frequency threshold of 0.4 were used. Graphics were generated with MAPCHART 2.2 (Voorrips, 2002).

2.5. QTL analyses

Given the high increase of genetic markers in DF family and the relevance of sex determination in this species, we considered it important to refine QTL identification and marker association in the DF family to sex (Martínez et al., 2009). We followed a linear regression method implemented in the GridQTL program (www.gridqtl.org.uk; Seaton et al., 2006) using the SPportlet, as in our previous work (Martínez et al., 2009). Two approaches were employed: first, a single QTL was assumed at each LG; secondly, a two-QTL model was tested within each LG. GridQTL implements a linear regression methodology, considering the linkage phase between markers according to pedigree information. The Haseman and Elston (1972) method for QTL linkage analysis was applied, and the chromosome-wide significance thresholds at $P = 0.05$ and 0.01 were estimated with a permutation test of 10,000 iterations. An outbred full-sib model was used and a QTL was considered suggestive when significance was between 5% and 1% at chromosome-wide level and significant when significance was below 1% at chromosome-wide level or when significance was below 5% at genome-wide level. These thresholds also allowed establishment of an interval surrounding the most probable position where a significant signal was detected. Association between markers and phenotypes were investigated using ANOVA analyses. Each ANOVA also provided a corrected R^2 value useful to estimate the reduction of the overall phenotypic variance of the trait because of the model fitting, thus providing the proportion of the phenotypic trait variance explained by a single marker. A simple Bonferroni correction

per linkage group was also applied to avoid false positives from multiple testing.

2.6. Comparative mapping

Turbot gene sequences from Viñas et al. (2012) and Rodríguez-Ramilo et al. (2013), and genomic sequences by Ruan et al. (2010) were compared by BLASTn against updated versions of model fish genomes downloaded from ftp://ftp.ensembl.org: *Tetraodon nigroviridis* v.8.61, *Takifugu rubripes* v.5, *Danio rerio* Zv9.6, *Oryzias latipes* v.1.61 and *Gasterosteus aculeatus* v.1.61. BLAST searching was performed using a minimum alignment length of 40 bp with a percentage identity >80% as recommended for EST mapping across species and E-value threshold ($E < 10^{-5}$).

3. Results

3.1. The integrated turbot genetic map

The new compiled turbot map contains 487 markers (380 framework), ~40% EST (gene)-linked and ~60% anonymous (Tables 1 and S1; Figs. 1 and S1). Only 27 markers remained unlinked and 26 were accessory (they could be allocated to the nearest marker in a particular linkage group, but not ordered with confidence). The integrated map measured a total length of 1414 cM (framework: 1274.4 cM) and the genomic coverage was very close to 90% in both cases according to the estimated genomic lengths (Table 1). Average inter-marker distances were 3.3 and 3.6 cM for the total and framework maps, respectively.

The correspondence between linkage groups of the TB and TR maps is presented in Table 2 and Fig. S1. As expected, given the high number of linkage groups in the female TR map (30), several linkage groups of this map merged into a single one in the TB map, thus being reduced from 30 to 23 linkage groups. All the 21 male TR groups showed a univocal relationship with TB groups, although the LG20 and LG24 TB groups had no counterpart on the male TR map. Finally, the TB LG18 was not represented in either the female or the male maps.

3.2. Refinement of sex-associated QTL

Because the number of markers in the DF family greatly increased, we judged it interesting to re-evaluate associations between markers and sex due to the interest of sex determination in this species. Screened map length increased from 1074.9 cM to 1392.7 cM and inter-marker distance was lowered from 6.8 cM to 4.9 cM, thus representing a higher resolution panel.

We mapped all available markers from Ruan et al. (2010) at the counterparts of LG5 (M15 and F22: 3 markers) and LG21 (M12 and F18: 5 markers), and integrated previous mapped candidate genes related to gonad differentiation by Viñas et al. (2012). As in the work by Martínez et al. (2009), LG5 showed the most significant sex-related QTL, the SmaUSC-30 marker explaining up to 86.1% of the phenotypic variance (Table 3).

The other three sex-associated QTL detected by Martínez et al. (2009) were confirmed in this study, but two of them moved from suggestive to significant (LG6 and LG8) and in the case of LG8, the marker Sma-USC194 showed significant association explaining up to 16.1% of phenotypic variance. LG21, although increasing association figures, remained as suggestive.

Table 1
General statistics of the integrated turbot (*Scophthalmus maximus*) map.

	Consensus
Total markers	514
Mapped markers	487
EST (gene)-linked	190
Anonymous	297
Framework markers	380
EST (gene)-linked (framework)	123
Anonymous (framework)	257
LOD < 3 markers	71
Accessory	36
Unlinked	27
Total length	1411.50 (89.9%)
Maximum intermarker distance	28.2
Mean intermarker distance	3.3
Framework length	1274.4 (88.1%)
Maximum intermarker distance	28.2
Mean intermarker distance	3.6
Genome length (total)	1572.4
Genome length[a] (framework)	1447.2

In parentheses: percentage of genomic coverage of turbot map.
[a] Genome length was estimated according to Hubert and Hedgecock (2004).

3.3. Comparative mapping

As expected, all sequences of sex-related genes rendered significant hits when compared with model fish species (Table S2). However, only 16 microsatellite markers from Ruan et al. (2010) showed significant hits (10.1%). Among these 16 anonymous markers, three were mapped in the integrated map, and thus consistent syntenies could be established with model fish genomes. For the remaining ones, only predictive positions could be established using previous macrosyntenies reported by Bouza et al. (2012).

Finally, some unlinked genes in the TR map (YSKr161 at LG9, YSKr255 at LG13 and YSKr27 at LG8) could be associated to specific linkage groups by predictive mapping.

A consistent macrosyntenic pattern had been reported between turbot and Acanthopterygii model fish genomes (stickleback [Gac], medaka [Ola], fugu [Tru], tetraodon [Tni]) (Bouza et al., 2012). This pattern was confirmed for the 14 significant hits in this study, and only one gene (*Sox19*) matched consistently with three model fish genomes (Gac, Ola, Tru) to a different chromosome than that predicted.

Within the refined confidence interval of the sex-QTL at the LG5 proximal region, very low homology was detected. Only SmaUSC-E30 and SmaSNP_31 showed significant homology against stickleback genome at closely linked positions in GacVIII (Table S2), concordant with matches of LG5 against orthologous chromosomes in other models (Tni1, Ola4 and Tru20; Bouza et al., 2012; Martínez et al., 2009). Gene mining between these syntenic positions at GacVIII using BioMart-Ensembl revealed two genes related to reproductive developmental process (GO term 0003006): *DNAJC19* and *Sox2* (sex determining region Y-box 2) located in the vicinity at GacVIII (Table S2).

4. Discussion

This work represents the compilation of several genetic mapping resources of turbot, resulting in a new linkage genetic map with 380 framework markers out of 487 total mapped markers. Besides the increased number of markers, this map represents the integration of the two main genetic maps previously reported in this species (Bouza et al., 2012; Ruan et al., 2010). The number of new markers allowed refinement of the position of previously mapped markers, since linkage information greatly increased. Accordingly, six of the 39 previous accessory markers could be mapped, and 13 markers previously mapped with LOD <3.0 were now positioned as framework. Increased marker information resulted in a decrease of inter-marker distances. Average inter-marker distances were 3.3 and 3.6 cM for the total and framework maps, respectively, which represent physical distances of 1.4 and 1.7 Mb, respectively, according to the estimated turbot genome length (Cuñado et al., 2001). Maximum distance between markers was below 20 cM at all linkage groups (mean 12.7) excluding LG12 (28.2 cM). All these figures show a significant improvement compared with previous turbot maps (Bouza et al., 2012; Ruan et al., 2010).

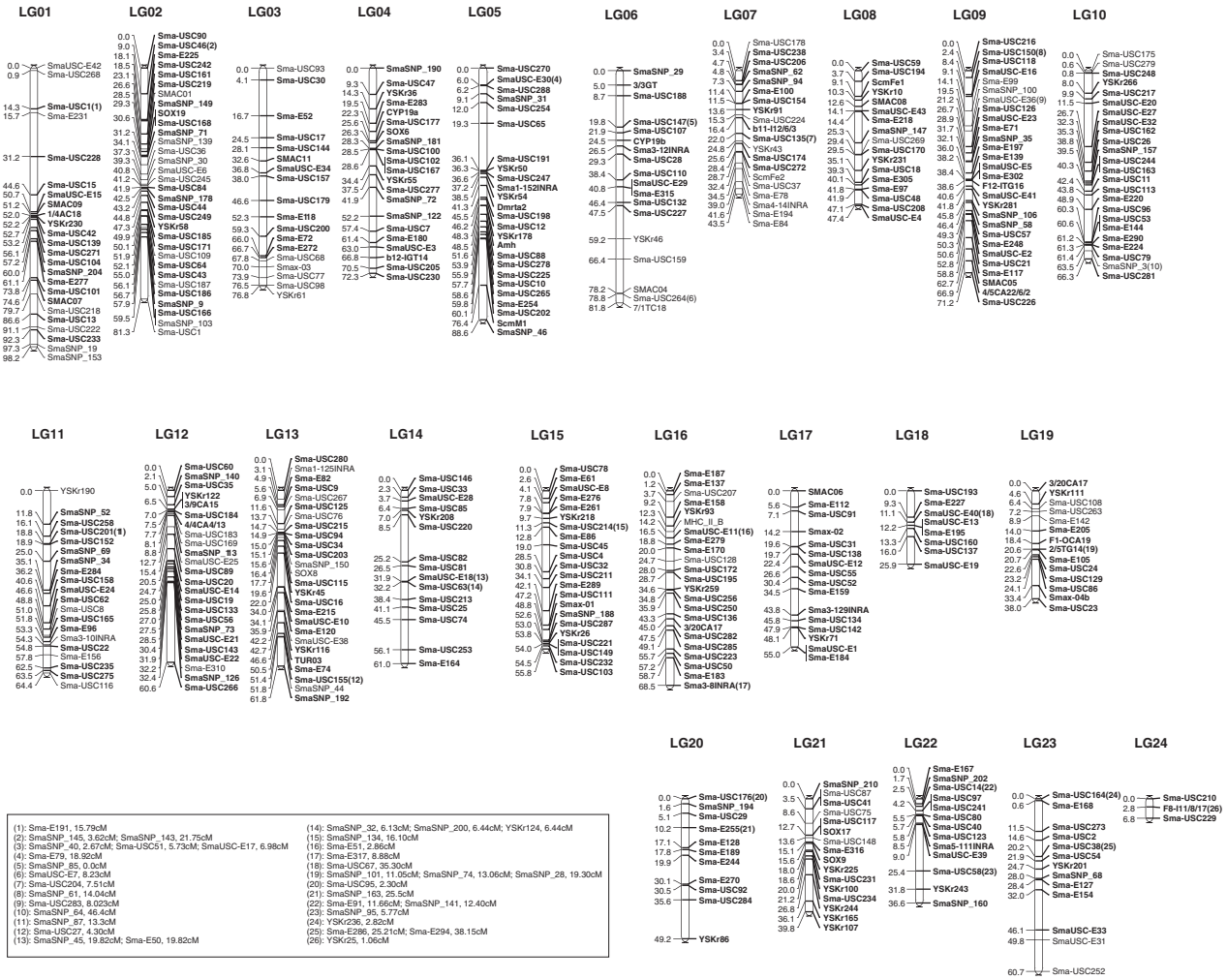

Fig. 1. Integrated consensus turbot map. Framework markers in bold characters; accessory markers in parentheses beside the closest marker and listed in the square of the figure; LOD < 3 markers in normal type.

Table 2
Statistics per linkage group of the turbot (*Scophthalmus maximus*) consensus genetic map.

LG	LG (TR)	M	Frw M	LOD < 3.0	Acc.	L	D	D_M	D_m	Frw L	fD	fD_M	fD_m
LG01	M11/F12	25	17	7	1	98.2	4.3	15.5	0.4	78.0	4.9	16.9	0.4
LG02	M1/F1	37	22	10	5	81.3	2.6	21.8	0.0	59.5	2.8	10.7	0.0
LG03	F14/M21	18	12	6	0	76.8	4.5	12.5	0.3	62.6	5.7	12.5	0.7
LG04	M9/F29 & F6	21	21	0	0	72.3	3.6	10.3	0.0	72.3	3.6	10.3	0.0
LG05	M15/F22	26	25	0	1	88.6	3.7	16.8	0.1	88.6	3.7	16.8	0.1
LG06	M13/F13	20	13	5	2	81.8	4.8	11.8	0.0	47.5	4.0	11.1	0.0
LG07	M8/F21 & F16	21	11	9	1	43.5	2.3	5.6	0.1	25.0	2.5	5.6	0.1
LG08	M3/F17 & F26	17	16	1	0	47.4	3.0	10.9	0.1	47.4	3.2	10.9	0.1
LG09	M6/F3	30	25	3	2	71.2	2.6	6.0	0.2	71.2	3.0	17.6	0.4
LG10	M4/F5	25	21	3	1	66.3	2.9	15.2	0.0	65.5	3.3	15.2	0.0
LG11	M2/F2	21	15	5	1	64.4	3.4	11.8	0.1	51.7	3.7	10.1	0.1
LG12	M10/F27	24	20	4	0	60.6	2.6	5.1	0.0	60.6	3.2	28.2	0.3
LG13	M14 & F28	27	19	7	1	61.8	2.5	12.0	0.1	61.8	3.4	12.0	0.1
LG14	M19/F9 & F19	20	15	0	5	61.0	4.4	16.8	0.4	61.0	4.4	16.8	0.4
LG15	M5/F4 & F23	23	22	0	1	55.8	2.7	9.5	0.0	55.8	2.7	7.9	0.0
LG16	M7/F15 & F7	25	20	3	2	68.5	3.1	9.8	0.2	68.5	3.8	9.8	0.2
LG17	M16/F10	16	16	0	0	55.0	3.7	9.3	0.0	55.0	3.7	9.3	0.0
LG18	–	9	8	0	1	25.9	3.7	9.9	0.1	25.9	3.7	9.9	0.1
LG19	M20/F11	17	11	3	3	38.0	2.9	9.3	0.7	38.0	3.7	9.4	0.2
LG20	F30	13	11	0	2	49.2	4.9	13.7	0.4	49.2	4.9	13.7	0.4
LG21	M12/F18	16	13	3	0	39.8	2.7	9.3	0.0	39.8	3.3	9.3	0.0
LG22	M17/F24	16	13	0	3	36.6	3.1	16.5	0.1	36.6	3.1	16.5	0.1
LG23	M18/F8 & F20	16	11	2	3	60.7	5.1	14.1	0.4	46.1	4.6	14.1	0.4
LG24	F25	4	3	0	1	6.8	3.4	4.0	2.8	6.8	3.4	4.0	2.8
Total		487	380	71	36	1411.5				1274.4			
Mean		18.8	15.8	2.9	1.5	58.8	3.3	11.6	0.3	53.1	3.7	12.4	0.3

LG: linkage group; TR: turbot genetic map by Ruan et al. (2010); M: number of markers; Acc.: Accessory markers; L: length; D: mean intermarker (IM) distance; D_M: maximum IM distance; D_m: minimum IM distance; fD: framework IM distance (all IM distances in cM).

This map integrates the resources of the two main turbot maps by Bouza et al. (2012; TB) and Ruan et al. (2010; TR), plus 13 additional gene markers associated with gonad differentiation (Viñas et al., 2012) and one with immunity (Rodríguez-Ramilo et al., 2013) (Table S1). Most new markers were located using the main mapping family (DF: 38 markers), now including 319 markers. The correspondence between the TR and TB maps was established because, as expected, the 31 selected markers from all different TR linkage groups were associated to one linkage group in the TB map (Table 2, Fig. S1). Thus, the integrated turbot map potentially contains 592 markers (487 mapped + 105 not mapped from TR) given the correspondence established between both maps. This number of markers would nearly represent 1 marker/Mb according to the reported turbot genome by Cuñado et al. (2001).

All linkage groups of the TR map found its counterpart in the TB map (Fig. S1). The 21 male TR linkage groups showed a univocal relationship to one of the TB linkage groups, while several TR groups of the female map converged into only one TB group, as expected because of its high linkage group number. As previously reported (Bouza et al., 2012), both segregation and comparative mapping data strongly suggest that LG18 is linked to LG8 in the TB map. Based on this information, the integrated turbot map would include 23 linkage groups, only one above that expected from the turbot karyotype (n = 22) (Bouza et al., 1994).

Table 3
Refinement of the sex-associated QTL analysis in turbot (*Scophthalmus maximus*).

LG	EP	I	Sig.	Associated marker	Marker position (cM)	R^2 (%)
5	**0**	**0–12**	*	**SmaUSC-E30**	**0.0**	**86.1**
	0	0–30	*	SmaUSC-E30	6.0	86.1
				Sma-E79	25.3	36.0
				YSKr50	36.3	17.5
				Sma-USC247	36.6	17.8
				Sma1-152INRA	37.2	17.0
				YSKr54	38.5	16.5
				Sma-USC225	55.9	19.9
				Sma-USC10	57.7	14.1
6	**20**	**15–30**	**s**			
	34	32–36	*			
8	**62**	**58–65**	**s**			
	7	6–8	*	Sma-USC194	3.7	16.1
21	**18**	**0–19**	**s**			
	14	0–24	s			

LG: linkage group; EP: estimated position (in cM); I: interval of the detected QTL (in cM); Sig.: significance level. QTL can be suggestive (s), or significant (*); R^2: percentage of the total phenotypic variance explained by the marker according to the model. In bold: information obtained from Martínez et al. (2009).

Increasing marker density simplifies the identification of candidate genes as a consequence of refinement of the position where the QTL is detected. It also allows discounting of false-positives in association studies, because the correction for multiple tests is more restrictive, therefore increasing confidence in the detected associations (Collard et al., 2005). The increased marker density gave us the chance to reanalyze marker associations with sex. The number of significant associated markers greatly increased at the main sex-related QTL, and thus its interval widened from 0–12 to 0–30 cM. This was likely due to the reallocation of the most significant associated marker (see Table 3), previously positioned at 0.0 cM and now at 6.0 cM in the new map. Sex-associated reanalysis confirmed the main results reported by Martínez et al. (2009), but the statistical confidence was increased to significance in two of the minor QTL at LG6 and LG8. Although the statistical significance of LG21 increased, it remained as suggestive despite the interesting features of this linkage group related to sex, such as the presence of two relevant Sox genes (*Sox9* and *Sox17*) and the nearly complete lack of recombination in males (Bouza et al., 2012). *SRY*-related high-mobility-group box (*Sox*) genes constitute a family that encodes transcription factors related to gonad differentiation. Both *Sox9* and *Sox17* are reportedly related to testis differentiation at the beginning of development and thus, represent putative candidates related to sex determination in this species (Viñas et al., 2012). Also, recombination restriction is considered an important step in the evolution of sex chromosomes to avoid breaking the association between the sex-determining gene and favorable allelic variants at antagonistic genes (Bergero and Charlesworth, 2009). LG21 is the only turbot linkage group where recombination frequency differences between males and females are extreme (Bouza et al., 2012). On the other hand, the two sex-determining candidate genes on LG5 (*Dmrta2* and *Amh*) mapped far away from the main associated marker,

SmaUSC-E30, and as suggested by Viñas et al. (2012), they appear not to be the master sex-determining gene in turbot. Our results confirmed the existence of a major sex-determining locus at LG5 in turbot, but also suggest more consistently the existence of other minor genetic factors associated to LG6, LG8 and LG21 involved in sex determination in this species.

Only 16 microsatellite markers (10.1%) from Ruan et al. (2010) gave significant hits with model fish genomes, a circumstance explained both by their anonymous condition, which determines higher evolutionary rates than coding sequences (Bouza et al., 2012), and by the short length of adjacent microsatellite sequences available in GenBank. In fact, the percentage of successful hits was much lower than that observed by Bouza et al. (2007) also based on anonymous microsatellite sequences (22.7%). The consistent macrosyntenic pattern between turbot and Acanthopterygii model fish genomes, reported in Bouza et al. (2012), was confirmed for the 14 significant hits of new mapped markers in this study. Only one matched consistently to a different chromosome than predicted with three model fish genomes. Several genes showed significant secondary hits with other model fish chromosomes in addition to that expected according to macrosyntenic patterns, likely due to the existence of paralogous genes. Conversely, the predictive positions obtained through comparative mapping for non-mapped TR markers were mostly concordant with the correspondence obtained between the TB and TR maps.

In agreement with Martínez et al. (2009), little homology with model genomes was detected within the refined confidence interval of the sex-QTL (0–30 cM). Among the query markers, only SmaUSC-E30 and SmaSNP_31 showed significant homology with the stickleback genome, concordant with matches of LG5 against orthologous chromosomes in other models (Tni1, Ola4 and Tru20; Bouza et al., 2012; Martínez et al., 2009). Gene mining revealed two genes related to the reproductive developmental process: *DNAJC19* and *Sox2*. The latter is a relevant candidate for further marker development since the Sox family encodes transcription factors involved in a variety of embryonic developmental pathways, including genitalia development. The former, *DNAJC19*, has been shown to be related to sex-determination in teleosts and vertebrates (Cnaani et al., 2007; Mazzuchelli et al., 2011).

In conclusion, this study compiles all turbot mapping resources from different studies and integrates them into a single consensus map including 487 markers and 105 unmapped markers based on the correspondence established between the two main previously reported turbot maps. Reanalysis of sex-associated QTL with this consensus map allowed us to further support the existence of minor genetic factors involved in sex determination in addition to the main one located at LG5. Comparative mapping consolidated our previous view on conserved genomic macrosyntenies within Acanthopterygii, facilitated predictive mapping for unlinked markers in previous works and enabled investigation of candidate genes for sex determination.

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