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A radiation hybrid map of the European sea bass (*Dicentrarchus labrax*) based on 1581 markers: Synteny analysis with model fish genomes

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ABSTRACT

The selective breeding of fish for aquaculture purposes requires the understanding of the genetic basis of traits such as growth, behaviour, resistance to pathogens and sex determinism. Access to well-developed genomic resources is a prerequisite to improve the knowledge of these traits. Having this aim in mind, a radiation hybrid (RH) panel of European sea bass (*Dicentrarchus labrax*) was constructed from splenocytes irradiated at 3000 rad, allowing the construction of a 1581 marker RH map. A total of 1440 gene markers providing ~4400 anchors with the genomes of three-spined stickleback, medaka, pufferfish and zebrafish, helped establish synteny relationships with these model species. The identification of Conserved Segments Ordered (CSO) between sea bass and model species allows the anticipation of the position of any sea bass gene from its location in model genomes. Synteny relationships between sea bass and gilthead seabream were addressed by mapping 37 orthologous markers. The sea bass genetic linkage map was integrated in the RH map through the mapping of 141 microsatellites. We are thus able to present the first complete gene map of sea bass. It will facilitate linkage studies and the identification of candidate genes and Quantitative Trait Loci (QTL). The RH map further positions sea bass as a genetic and evolutionary model of Perciformes and supports their ongoing aquaculture expansion.

Introduction

Teleost fishes represent the most numerous and diversified group of vertebrates. Within this group, the order of Perciformes is by far the most numerous and highest evolved. European sea bass (*Dicentrarchus labrax*; family of Moronidae) is one of its biologically well-documented members and one of the more valuable marine finfish. The European aquaculture production amounted to 138,156 tons in 2008 (http://www.aquamedia.org/production/species/seabasses/seabassprod_en.asp?). Its expansion is a consequence of the development of techniques allowing the control of reproduction and growth from larva to portion size in the seventies and eighties [1].

However today's sea bass industry still suffers from some major constraints. There is limited access to selected lines, which may be attributed to poor acceptance of controlled mating. Sea bass breeders have essentially a wild genetic profile; they are experiencing poorly managed domestication selection and relaxation from natural selection pressure under aquaculture conditions [2]. Most appreciated by producers and consumers are traits related to growth, feeding efficiency, gender, disease resistance and immunity. Fortunately the heritability of many aquaculture traits is moderate to high [3], making the potential for selection high. It has resulted in single generation gains of 23% in growth in European sea bass [2]. Several QTLs involved in body weight, a multigenic trait, and shape have been identified in European sea bass [4]. But production losses in aquaculture remain huge due to infection by viruses and bacteria, and to a lesser extent by parasites. Enhancement of innate and acquired immunity through breeding, husbandry and vaccination is an acknowledged strategy to address this issue (see [5] for review). Also, considerable environmental and behavioural stresses are induced by the atypical aquaculture environment. Since stress has a genetic component, selection should be feasible [4,6]. While sex determination is polygenic in sea bass [7], sex differentiation is growth-dependent and environmentally tuned [8]. Hence gender control remains a big issue in sea bass breeding. Another challenge is the induction of sterility to optimise the production of portion-sized fish and to limit the environmental impact from escapees [9]. Of a more recent interest is the substitution of current fish food with plant ingredients for a more sustainable aquaculture [10]. This strategy may directly affect quality traits related to growth and metabolism, whose heritability is under investigation in sea bass [11].

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Hence, understanding the genetic basis of traits of interest will directly benefit the identification of the best breeders and the management of the broodstock. But the feasibility of these studies relies on the availability of well-developed genomic resources. The more established fish models such as zebrafish *Danio rerio*, spotted green pufferfish *Tetraodon nigroviridis*, fugu *Takifugu rubripes*, three-spined stickleback *Gasterosteus aculeatus* and medaka *Oryzias latipes* are of no aquaculture interest. However, a growing group of economically important fishes (e.g. Atlantic salmon *Salmo salar*, Nile tilapia *Oreochromis niloticus*, carp *Cyprinus carpio*, rainbow trout *Oncorhynchus mykiss*, channel catfish *Ictalurus punctatus*, gilthead seabream *Sparus aurata* and European sea bass) is being supported by genomic resources driven by important technological developments in molecular biology.

The sea bass genome recently underwent a 2× WGS sequencing combined with the alignment of both ends of ~45,000 BAC clones on the stickleback sequence [12]. Establishing dense and accurate maps for these species along with comparative data with other species makes it very attractive to extrapolate genome architecture, gene function, evolution and structure. Genetic linkage maps of the sea bass genome, consisting of 174 and 368 polymorphic markers, were constructed by Chistiakov et al. in 2005 [13] and 2008 [14], respectively. These maps provided a first characterisation of the genome and identified consistent low resolution synteny relationships with model fish species. The reason is that linkage maps are usually made with relatively few gene markers, which limit interspecific comparisons. By contrast, radiation hybrid (RH) mapping is suited to map all types of markers, it does not require locus-specific polymorphism, and it offers higher density mapping. RH mapping thus allows to construct comparative maps through the mapping of genes and the localisation of their orthologs in other species [15]. In fish genomics this was illustrated by the construction of two gene-based RH maps of the gilthead seabream genome and their correspondence with the spotted green pufferfish genomic sequence [16,17]. However synteny comparisons between seabream and sea bass, and between seabream and tilapia were made indirectly via comparisons with stickleback. Global synteny relationships between farmed Perciformes (seabream, sea bass and Nile tilapia) and model species (three-spined stickleback, medaka and spotted green pufferfish) [18] fit with the generally accepted phylogeny based on the mitogenome [19] and 42 orthologous genes [20].

High resolution physical maps such as RH maps are also of great help in the assembly of sequencing data in shotgun projects [21]. Indeed, in many cases "finished" genome sequences from shotgun projects contain large sequence gaps that imply inconsistencies in the positioning of scaffolds [22]. In addition, low-depth sequences lack long-range continuity and provide only a fragmented view of a genome. This is exemplified by the fugu genome sequence made of 7213 unconnected scaffolds without any chromosome assignment [23].

Still, the construction of RH panels of fish species has turned out to be more difficult than in mammals, where most RH panels have been developed (see [15] for review). To date, only three fish RH panels have been reported: two RH panels of zebrafish derived from stable cell lines [24,25] and one RH panel of seabream derived from primary fibroblasts [16]. Here we report for the first time the production of fish radiation hybrid lines from splenocytes. The sea bass RH panel was used to construct a 1581 marker RH map of the sea bass genome. The mapping of 1440 gene markers designed from ESTs and complete mRNA allowed to establish gene order along the 24 sea bass chromosomes and to identify synteny relationships with other fish model genomes. Moreover, the mapping of 141 microsatellites made it possible to integrate the sea bass genetic linkage map in the RH map. It is the first dense RH map of the sea bass genome obtained from a high resolution panel with good performance. It is also the first complete gene map of European sea bass.

Methods

Selection of radiation hybrid cell lines

Juvenile sea bass of average weight 50 g were kindly provided by Ifremer (Brest, France) and kept at the INRA, SCRIBE (Rennes, France) fish husbandry facility until needed for fusion experiments. When proceeding with the fusion experiment, fish were euthanised with an overdose of 2-phenoxyethanol and rinsed briefly in 70% ethanol. The spleen was rapidly dissected and rinsed several times in washing medium (Leibovitz L-15; 1×Penicillin/Streptomycin/Fungizone). The spleen was cut in small pieces and fragmented between the frosted edges of glass slides. The obtained cell suspension was ready for irradiation. A panel of radiation hybrid cell lines was constructed using the methodology described previously [26]. Briefly, the sea bass spleen cell suspension was irradiated by a 3000-rad γ-ray exposure. Splenocytes were fused with $HPRT^-$ derivative CHO cells in a 5:1 ratio in the presence of polyethylene glycol 1500 (Roche, Mannheim, Germany). Following selection in HAT medium and minimum cell culture expansion (3 to 4 weeks), DNA was extracted from individual clones (QIAmp DNA kit, protocol for cultured cells; Qiagen, Hilden, Germany).

DNA amplification

Once hybrid cell lines were selected on quantitative and qualitative criteria, their DNA was expanded by Whole Genome Amplification (WGA) using φ29 DNA polymerase of V2 and HY GenomiPhi kits (GE healthcare, Fairfield CT, USA). DNA concentration was first estimated with a Nanodrop (Thermo Scientific, Wilmington DE, USA) for every cell line. A first step consisting of two separate WGA was performed from 2×10 ng of DNA with the V2 GenomiPhi kit. WGA products were pooled to constitute the stock panel. A second step consisting of two separate WGA was performed with the HY GenomiPhi kit from 2×10 ng of DNA from the stock panel. Products were pooled to constitute a working panel allowing to genotype several hundreds of markers. When needed, additional material was prepared by repeating the second step. The WGA reliability was previously demonstrated by Senger et al. [16].

Marker definition

All sea bass ESTs and mRNAs available at the time of the study were downloaded from the GenBank database (April 2009). Sequences were masked for simple repeats with the RepeatMasker web server (http://www.repeatmasker.org/) and aligned together with the CAP3 software [27] in order to build contigs of overlapping sequences. Default parameters of the CAP3 software were used for the assembly step.

In order to identify orthologous sequences between sea bass and four model fishes (three-spined stickleback, medaka, pufferfish and zebrafish), the contigs and singlets obtained from the assembly step were aligned to the respective genome sequences using the Exonerate v1.4.0 software [28]. Sea bass sequences having a hit with a minimal score of 250 and an alignment size of 80 to 300 bp with model species genomic sequences were selected and further used to design markers. PCR markers of 60 to 150 bp long with primers of 23 nt average length were designed using the Primer3 v0.4.0 software [29]. For each of the sea bass sequences the coordinates of the best hit on each of the model fish genomes were considered as the location of the putative orthologs. Orthologous genes with gilthead seabream were also identified by alignment of sea bass sequences to the seabream sequences mapped by Sarropoulou et al. [17]. Hits between sea bass and seabream sequences were selected using the same criteria as above, except that no maximal alignment size was imposed, as sequences of both species consisted of ESTs.

Orthologs 1:1:1:1:1 between sea bass, stickleback, medaka, pufferfish and zebrafish were used to estimate gene sequence conservation

between these species. All hits localised between coordinates of these orthologs as annotated by Ensembl were identified and a conservation coefficient between sea bass and each species was calculated as follows: coeff = ∑(identity_% × identity_length)/∑(identity_length). The Ensembl gene coordinates for each species were downloaded from the Biomart webserver (http://www.biomart.org).

Genotyping

PCR were performed on 50 ng of DNA in a final volume of 10 μl and at a final concentration of 0.3 μM of each primer, 250 μM of each dNTP, 1.5 or 2 mM $MgCl_2$, 50 mM KCl, 10 mM Tris–HCl, plus 0.05 U of AmpliTaq Gold polymerase (Applied Biosystems, Foster city, CA). Reactions were carried out in MJ (MJ Research, Cambridge, MA) or GeneAmp 9700 (Applied Biosystems) thermocyclers with the following PCR program: 1 inducing step of 95 °C for 8 min; 20 cycles of 94 °C for 30 s, 61 °C (or 57 °C) for 30 s (−0.5 °C/cycle), 72 °C for 1 min; 15 cycles of 94 °C for 30 s, 51 °C (or 47 °C) for 30 s, 72 °C for 1 min; 1 final extension step of 72 °C for 2 min.

PCR products were analysed by electrophoresis in a 2% agarose gel in 0.5× TBE buffer. Gel images were recorded with a high resolution CCD camera (Vilber Lourmat, Torcy, France). Results were scored as present, absent or ambiguous in a semi-automated fashion. Microsatellite markers mapped on the first generation linkage map [13] were also genotyped.

Data computation

A two-point analysis was performed using the Multimap v2.0 software [30], starting at a lod score of 3.5. The multipoint analysis was performed with the CarthaGène v1.0 software [31]. RH groups that harboured obvious aberrations were re-analysed at higher two-point lod scores of up to 4.5, before performing the multipoint analysis again. Distances between markers were expressed in centirays (cR_{3000}). Coordinates of the putative orthologous genes retrieved from the Exonerate analysis of sea bass sequences with the four model genomes were aligned with the corresponding sea bass markers on the graphic representation. Ordered RH groups were tentatively oriented according to two-point lod scores between their end-markers. The microsatellite order on the linkage map and the synteny relationships with the four model species were compared with the RH map. Conserved Segments (CS) and Conserved Segments Ordered (CSO) between sea bass and the model species were identified using the AutoGRAPH web server [32]. A default adjacency penalty of 5 was used for the counting of CSO.

Results and discussion

Radiation hybrid panel

Sea bass splenocytes were γ-irradiated with a 3000 rad dose, fused with $HPRT^-$ derivative CHO host cells and selected on HAT medium as described in the Methods section. A total of 290 hybrid cell lines was obtained through three fusion experiments. The retention frequency was estimated for every clone by typing a set of 96 microsatellite markers taken from the genetic map [13] and by scoring their presence/absence pattern. The retention frequency of the 96 microsatellite set in every clone is shown in Fig. 1. The average retention frequency of the 290 clones was 19.8%.

A subset of 93 hybrid cell lines was selected to constitute the RH panel. Hybrid cell lines with high retention frequency were selected with the highest priority. However hybrid cell lines exceeding 50% retention were not selected in order to normalise the retention frequency of the hybrid lines of the RH panel. These cell lines were replaced by other cell lines, taking into consideration the retention value of each individual marker to get a better representation of the whole genome.

The average retention frequency of the RH panel is 30.6%. This value corresponds to the average amount of the sea bass genome retained in each clone as well as to the chance that a given marker is present in a given clone. Retention frequency of the clones ranged from 17% to 49% with 73% of the clones ranging from 25 to 40% retention. Finally, 94 of the 96 microsatellites are present in more than 5% of the hybrid cell lines.

A WGA with φ29 DNA polymerase was subsequently performed from a DNA aliquot of each cell line of the panel. To our knowledge this is the first time that a RH panel is constructed from fresh cells requiring no primary culture. Moreover this cell type is convenient to use as the dissociation of the spleen is performed in a relatively short time preceding the irradiation. By doing so, the chromosomal rearrangements that frequently occur in long-term cultured cell lines were avoided. The WGA reliability and its assets upon cell culture expansion to generate a large amount of DNA were discussed previously in Senger et al. [16].

Marker identification

Sequences of 44,358 ESTs and 399 mRNAs were downloaded from GenBank (April 2009), excluding mitochondrial sequences. Once simple repeats were masked, the assembly with the CAP3 software [27] with default parameters resulted in 7495 contigs and 13,371 singlets for a total of 20,866 unique sequences. Alignments with the Exonerate software [28] with the criteria described above revealed that 12,882 (~62%) of these sequences had a hit in the stickleback genome, 10,488 (~50%) in the medaka genome, 10,402 (~50%) in the pufferfish genome, and 7093 (~35%) in the zebrafish genome. A Venn diagram recapitulates how hits are assigned to the four model genomes, pointing to 6305 sequences shared by all of them (Fig. 2). Coordinates of the corresponding genes in the genomic sequences were retrieved based on the Ensembl gene annotation for each model species. All hits localised within these coordinates and putatively corresponding to exons of the orthologous genes were identified. Global sequence conservation between sea bass and the model species was assessed as described in the Methods section. The identity coefficient was estimated to 83.6% with stickleback, 81.1% with medaka, 79.4% with green spotted pufferfish and 76.1% with zebrafish. According to these values the stickleback is evolutionarily the closest to the sea bass, and the furthest from the zebrafish while distances with medaka and pufferfish are intermediate. Although the values may be biased as the sequencing completion of the model genomes is unequal, these results are consistent with phylogenies previously observed by Kuhl et al. [12] when aligning BAC-end sequences on the four model fish genomes. The decreasing proximity of sea bass with stickleback, medaka, pufferfish and zebrafish is also consistent with the phylogenetic gene trees constructed from the genes SOX17 [33] and IFN [34]. Trees made with seabream and the model species show a similar phylogeny [18,35].

EST-based markers were designed in priority from sequences conserved among stickleback, pufferfish and medaka. Based on sequence alignment scores, 1440 gene markers were selected, of which 1373 (95%) were conserved in stickleback, 1144 (79%) in medaka, 1132 (79%) in pufferfish and 758 (53%) in zebrafish. The Ensembl identifiers of orthologous genes in each species are listed in Supplemental Table 2 (S2). Finally, 1420 markers (99%) were conserved in at least one fish model species. Of those 1420 markers, 702 (49%) allowed anchorage between sea bass and the four species, 341 (24%) with three of them, 199 (14%) with two and 178 (14%) with only one model species, accounting for a total of 4407 anchors. However not all of those orthologous markers provided true anchors with the genomes of the fish model species, as the availability of coordinates depends on the assembly status of the genome sequence.

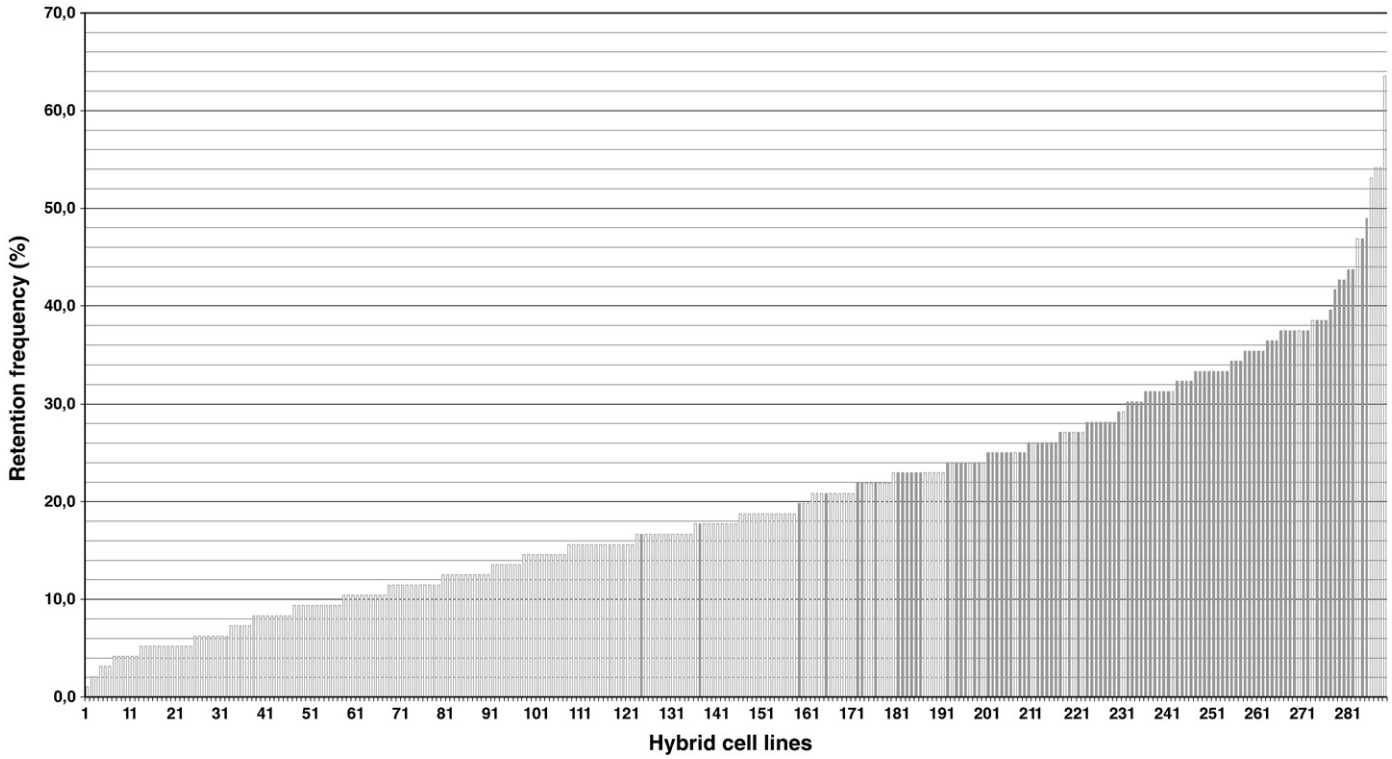


Fig. 1. Retention frequency of the sea bass hybrid cell lines. Hybrid cell lines are numbered from 1 to 290 on the X axis. Their retention frequency, estimated by PCR determination of the presence/absence of 96 microsatellite markers spread all over the sea bass genome [13], is represented on the Y axis. The 93 hybrid cell lines selected on qualitative and quantitative criteria that constitute the sea bass RH panel are figured in dark grey.

Thus 66 gene markers had orthologs identified on unlocalised scaffolds ("chromosome unknown") of the stickleback genome sequence, 351 markers aligned with the "chromosome unknown" of the pufferfish genome sequence, 135 markers aligned with the "chromosome unknown" of the medaka genome sequence and 55 markers aligned with unlocalised scaffolds of the zebrafish genome sequence. These proportions are in accordance with the proportion of the sequence assemblies assigned to chromosomes for the model species [36,37].

A score of 250 with the Exonerate software corresponds to a minimal alignment size of 50 bp, which is consistent with usual recommendations for mapping orthologous ESTs [38]. The maximal alignment size of 300 bp was imposed to avoid hits actually corresponding to retro-pseudogenes that may be mistaken for true orthologs. This value was based on the average size of exons estimated to be ~180 bp in the pufferfish [36]. The synteny conservation with the stickleback in the assigned RH groups for 933 markers out of 974 proved that these criteria were consistent, even though they are less efficient for identifying true orthologs in phylogenetically distant species like zebrafish.

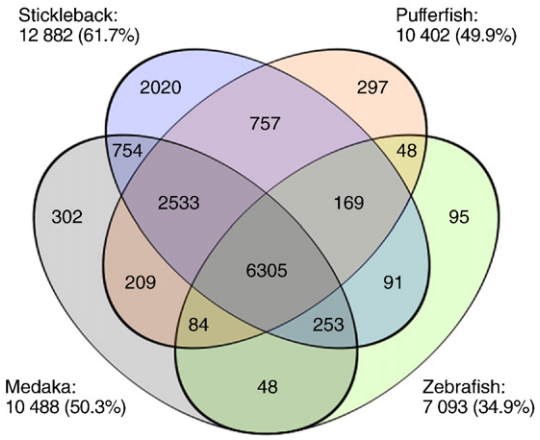


Fig. 2. Distribution of hits between sea bass and stickleback/medaka/pufferfish/zebrafish represented as a Venn diagram. Each model species is represented by an ellipse. The number of hits shared by two species or more are indicated in every intersection. For each model species, the number of sea bass sequences having one hit or more and their percentage of the total number of sequences analysed are indicated.

Characteristics of the RH map

The sea bass RH map contains a total of 1581 markers consisting of 1440 genes and 141 microsatellites. Assuming the 763 Mb size estimate of the sea bass genome [39] is correct, this represents a density of one marker per 480 kb. The typing data were analysed using the Multimap and CarthaGène softwares in an approach that minimised the number of RH groups and maximised their size while avoiding illegitimate fusions of RH groups.

The two-point analysis performed at a lod score of 3.5 resulted in 46 RH groups. As some of them harboured obvious aberrations, increasing lod scores were applied in a step-wise fashion up to the breaking point. Markers responsible for inappropriate fusions were also removed. Finally, 91 RH groups were obtained from two-point analyses performed at lod scores of 3.5 to 4.5. Characteristics of RH groups in terms of size, number of markers and associated orthology are shown in Table 1.

Data processing revealed 1552 markers (98%) with unique positions and 25 positions with two to four co-localised markers. These 91 RH groups with more than three markers represented 1342 markers, while 239 markers remained unlinked or in small groups of just two markers. Sixty-five of the RH groups containing three to 74 markers were assigned to the 24 sea bass chromosomes with the help of the microsatellite positions on the genetic map and the two-point lod scores between end-markers. It amounts to an average of 2.7 RH

Table 1
Characteristics of RH groups.

RH group	No. of markers[a]	No. of positions	No. of genes[a]	Size (cR_{3000})	Lod score	Stickleback		Pufferfish		Medaka		Zebrafish	
						No. of anchors[b]	chr[c]	No. of anchors[b]	chr[c]	No. of anchors[b]	chr[c]	No. of anchors[b]	chr[c]
LOD3.5-RH13	19	18	17	723	3.5	16	7	13	7	9	14 (2)	10	21, 14, 10, 9, 12
LOD3.5-RH14	18	18	13	670	3.5	13	18	8	14	9	24	5	20, 23
LOD3.5-RH16	16	16	15	533	3.5	15	7 (1)	9	7	13	14 (18, 13)	6	14, 5, 24, 18, 15
LOD3.5-RH19	8	8	8	257	3.5	7	8 (14, 1)	1	(1)	2	(4, 12)	5	6, 21, 2
LOD3.5-RH21	7	7	6	205	3.5	6	18	5	14	6	24	4	20
LOD3.5-RH5	48	48	45	1879	3.5	43	11 (5, 14, 13, 12)	24	3 (18, 2, 9)	40	8 (9, 7, 12, 10, 1)	26	3, 8, 6, 24, 22, 1
LOD3.5-RH10	34	33	25	1420	3.5	21	5	17	2 (15, 3)	17	19 (16)	15	12, 3, 8, 24, 20, 17
LOD3.5-RH15	16	16	16	627	3.5	14	18 (11)	14	14, 3	14	24 (22)	9	20, 3, 17, 13
LOD3.5-RH17	15	15	13	631	3.5	12	11 (15)	2	(11, 10)	8	8 (7)	8	3
LOD3.5-RH18	9	9	8	329	3.5	7	10	0		5	11	3	24, 19, 17
LOD3.5-RH20	8	8	8	237	3.5	8	19	8	13	6	6	6	18, 25, 2
LOD3.5-RH22	6	6	6	248	3.5	5	18 (4)	2	14	2	24	0	
LOD3.5-RH23	6	6	6	197	3.5	3	7	4	7	3	14	4	21
LOD3.5-RH24	5	5	5	236	3.5	5	19	4	13	5	6 (3)	3	4, 25, 18
LOD3.5-RH25	5	5	4	221	3.5	4	3 (7)	0		2	17	2	2
LOD3.5-RH26	4	4	4	161	3.5	3	12	3	9	2	7	2	8
LOD3.5-RH27	4	4	3	98	3.5	0		2	18	0		2	22
LOD3.5-RH28	4	4	4	122	3.5	4	6	0		3	(15, 10, 1)	2	7, 1
LOD3.5-RH29	4	4	4	124	3.5	3	13 (14)	2	12	1	(9)	2	8, 5
LOD3.5-RH3	74	72	67	2899	3.5	61	8 (15)	36	1 (8, 13)	48	4 (1)	25	22, 20, 2, 8, 11, 10, 6, 21, 17, 16
LOD3.5-RH31	4	4	4	146	3.5	4	4	1	(15)	4	23 (22, 17)	2	4, 11
LOD3.5-RH32	4	4	4	166	3.5	3	16	0		4	21	4	9, 6
LOD3.5-RH33	4	4	3	175	3.5	2	13	2	12	2	(9, 8)	2	8, 3
LOD3.5-RH34	4	4	4	112	3.5	4	18	3	14	3	24 (15)	3	20, 12
LOD3.5-RH35	4	4	4	171	3.5	3	1	3	3	0		3	1, 25
LOD3.5-RH36	3	3	3	142	3.5	3	14	0		1	(12)	2	5
LOD3.5-RH37	3	3	3	127	3.5	3	19	2	13	3	6 (17)	3	7, 4, 1
LOD3.5-RH38	3	3	3	107	3.5	2	19	2	13	1	(6)	2	25, 18
LOD3.5-RH39	3	3	3	95	3.5	3	18	3	14	0		2	20
LOD3.5-RH40	4	3	3	13	3.5	2	19	2	13	2	6	1	25
LOD3.5-RH41	3	3	3	63	3.5	3	1 (4)	0		2	(18, 13)	1	15
LOD3.5-RH42	3	3	3	140	3.5	2	13	0		2	9	0	
LOD3.5-RH43	3	3	2	88	3.5	0		1	(11)	1	(5)	1	6
LOD3.5-RH44	3	3	3	93	3.5	0		0		0		2	8
LOD3.5-RH45	3	3	2	94	3.5	2	15	0		0		0	
LOD3.5-RH46	3	3	3	43	3.5	2	7	1	(15)	3	18	1	5
LOD3.5-RH9	34	34	30	1338	3.5	27	7	3	20 (18)	22	18 (17)	10	7, 1
LOD3.6-RH28	7	7	7	209	3.6	7	9	1	(16)	2	1	3	1
LOD3.6-RH6	43	42	42	2025	3.6	34	15 (7, 18)	32	10 (14)	32	22 (11, 24, 7, 14, 1)	26	17, 2, 20, 19, 6, 24, 13
LOD3.6-RH10	25	25	21	1071	3.6	20	7 (1)	10	7	14	14 (13, 18)	12	10, 5, 15, 24, 21, 18
LOD3.6-RH12	23	22	21	1006	3.6	19	21 (20, 1)	11	6 (3)	20	20 (6, 2)	19	24, 2, 6
LOD3.6-RH13	19	19	18	905	3.6	17	19	11	13	11	6	10	25, 7, 18
LOD3.6-RH21	10	10	8	417	3.6	8	19	6	13	7	6 (11)	4	18, 17
LOD3.6-RH25	8	8	8	456	3.6	7	19 (20)	2	(5, 13)	3	6 (16)	3	25, 18
LOD3.6-RH29	6	6	6	273	3.6	6	4	2	(9, 13)	5	23	4	4, 7, 13
LOD3.6-RH38	4	4	3	126	3.6	0	0			2	20	2	24
LOD3.6-RH45	4	3	3	82	3.6	2	19	2	13	2	6	0	
LOD3.7-RH18	18	18	16	789	3.7	15	5	9	2 (9)	12	19 (7, 5)	9	12, 24, 20, 13
LOD3.7-RH52	3	3	2	49	3.7	1	(3)	0		1	17	0	
LOD3.7-RH11	25	25	24	1191	3.7	22	10 (9)	16	21 (20, 18)	15	11 (16, 1)	11	19, 16, 8
LOD3.7-RH47	4	4	4	121	3.7	2	(20, 10)	1	(17)	3	11	3	19
LOD3.8-RH15	30	30	30	946	3.8	28	20	20	8 (21)	24	16	15	16, 2
LOD3.8-RH27	17	17	17	882	3.8	17	20 (3)	13	8 (15)	10	16 (2, 17)	10	16, 20, 19, 1
LOD3.8-RH29	16	16	13	686	3.8	12	1	8	10, 16	9	13	7	15, 16, 1
LOD3.8-RH31	14	14	14	618	3.8	14	3	6	15	9	17	6	2, 24
LOD3.8-RH13	33	32	30	1327	3.8	29	4 (13)	19	1	21	10 (9)	19	14, 9, 8
LOD3.8-RH20	21	21	17	899	3.8	16	20	1	(8)	12	16	6	16
LOD3.8-RH22	20	20	20	724	3.8	20	15	14	10	17	22	9	17, 13, 21
LOD3.8-RH3	65	64	59	2761	3.8	54	14 (9, 4, 11)	23	4 (3)	46	12 (9, 8, 1)	33	21, 10, 5, 8, 3, 2, 19, 16, 12
LOD3.8-RH30	15	15	15	723	3.8	15	13 (5)	9	12	8	9	5	5, 21
LOD3.8-RH35	13	12	13	497	3.8	10	1	10	16	9	13	5	15, 5
LOD3.8-RH39	8	8	8	267	3.8	8	3 (6)	8	15 (9, 17)	7	17 (7)	7	2, 18, 24, 13
LOD3.8-RH57	5	4	5	108	3.8	4	1 (10)	4	16 (21)	2	13	1	15

Table 1 (*continued*)

RH group	No. of markers[a]	No. of positions	No. of genes[a]	Size (cR_{3000})	Lod score	Stickleback		Pufferfish		Medaka		Zebrafish	
						No. of anchors[b]	chr[c]	No. of anchors[b]	chr[c]	No. of anchors[b]	chr[c]	No. of anchors[b]	chr[c]
LOD3.8-RH6	43	43	36	2074	3.8	30	2 (4)	19	5	31	3	20	7, 18, 17, 6, 5, 25, 1
LOD3.8-RH67	3	3	3	86	3.8	3	5	2	2	2	19	1	14
LOD3.8-RH9	41	41	35	1689	3.8	33	3 (8, 10, 1)	23	15	33	17 (4, 13)	17	2, 20, 8, 4, 23, 22, 11
LOD3.9-RH3	53	52	49	2478	3.9	42	17 (15)	26	11 (9, 10)	38	5 (7, 22, 16)	30	6, 11, 23, 22, 8, 5, 4, 25, 21, 2, 19, 17, 1
LOD3.9-RH43	8	8	7	348	3.9	7	6	1	(17)	5	15 (18)	3	17, 12
LOD3.9-RH17	30	25	28	991	3.9	18	9	15	18	16	1 (12)	8	1, 6, 22, 21, 19
LOD3.9-RH47	7	7	7	223	3.9	7	16 (5, 1)	4	2 (3)	7	21 (2)	1	11
LOD3.9-RH50	6	6	6	205	3.9	6	19	5	13	6	6	3	4, 25
LOD3.9-RH52	6	6	6	258	3.9	6	20	5	8	4	16	3	16, 19
LOD3.9-RH73	3	3	3	82	3.9	2	12	1	(9)	2	7	1	23
LOD4-RH41	12	12	10	371	4	9	9 (2)	8	18 (9)	7	1	6	1, 14
LOD4-RH15	27	27	25	1413	4	24	16	19	2 (1, 17)	10	21 (7)	12	9, 6, 22, 16, 1
LOD4-RH49	8	8	8	217	4	1	(17)	6	11	3	5	6	6, 4, 12, 11
LOD4-RH52	7	7	7	309	4	7	13	3	12	5	9 (12)	1	
LOD4.1-RH14	27	26	27	1142	4.1	26	12	2	(9, 13)	14	7 (4)	18	23, 8, 7, 6, 4
LOD4.1-RH53	7	7	6	333	4.1	6	12 (11)	1	(19)	4	7 (8)	3	8, 23, 16
LOD4.1-RH59	6	6	6	275	4.1	6	4	3	19	4	23	1	4
LOD4.1-RH7	34	34	33	1406	4.1	32	6 (10)	21	17 (18)	24	15	17	13, 17, 20, 19
LOD4.1-RH82	4	4	4	79	4.1	4	1	4	3	3	2	2	9, 17
LOD4.2-RH11	30	29	28	1390	4.2	25	1 (21, 16)	16	3	15	2 (20)	12	1, 7, 6, 22, 9, 23
LOD4.2-RH22	20	20	20	814	4.2	20	10 (1)	7	21 (8, 16)	12	11 (17, 13)	11	19, 15, 5, 20, 1
LOD4.2-RH37	13	13	13	482	4.2	10	12 (19)	10	9 (19)	12	7 (23)	9	23, 6, 22, 11
LOD4.2-RH87	4	4	3	141	4.2	3	13	2	12	2	9	2	8
LOD4.4-RH3	40	39	39	1552	4.4	34	13 (15)	31	12 (4, 10)	26	9 (22)	22	8, 5, 21, 23, 13
LOD4.4-RH45	11	10	11	322	4.4	10	13	9	12 (4)	8	9	4	5, 17, 12
LOD4.4-RH54	7	7	6	233	4.4	2	16	6	2	5	21	4	1, 9
LOD4.5-RH21	17	17	16	702	4.5	15	12	9	9	12	7	8	8, 23, 6
LOD4.5-RH31	14	14	14	675	4.5	14	4 (3)	9	19	11	23	7	4
		1320	1237	54373		1104		682		869		639	

No distinction is made between conserved segments and singletons in the case of zebrafish.

[a] Including the co-localised markers. Note that co-localised markers are not taken into the account of anchors to model genomes.

[b] Only anchors connecting assigned scaffolds are taken into account.

[c] Number in parentheses correspond to singleton localization.

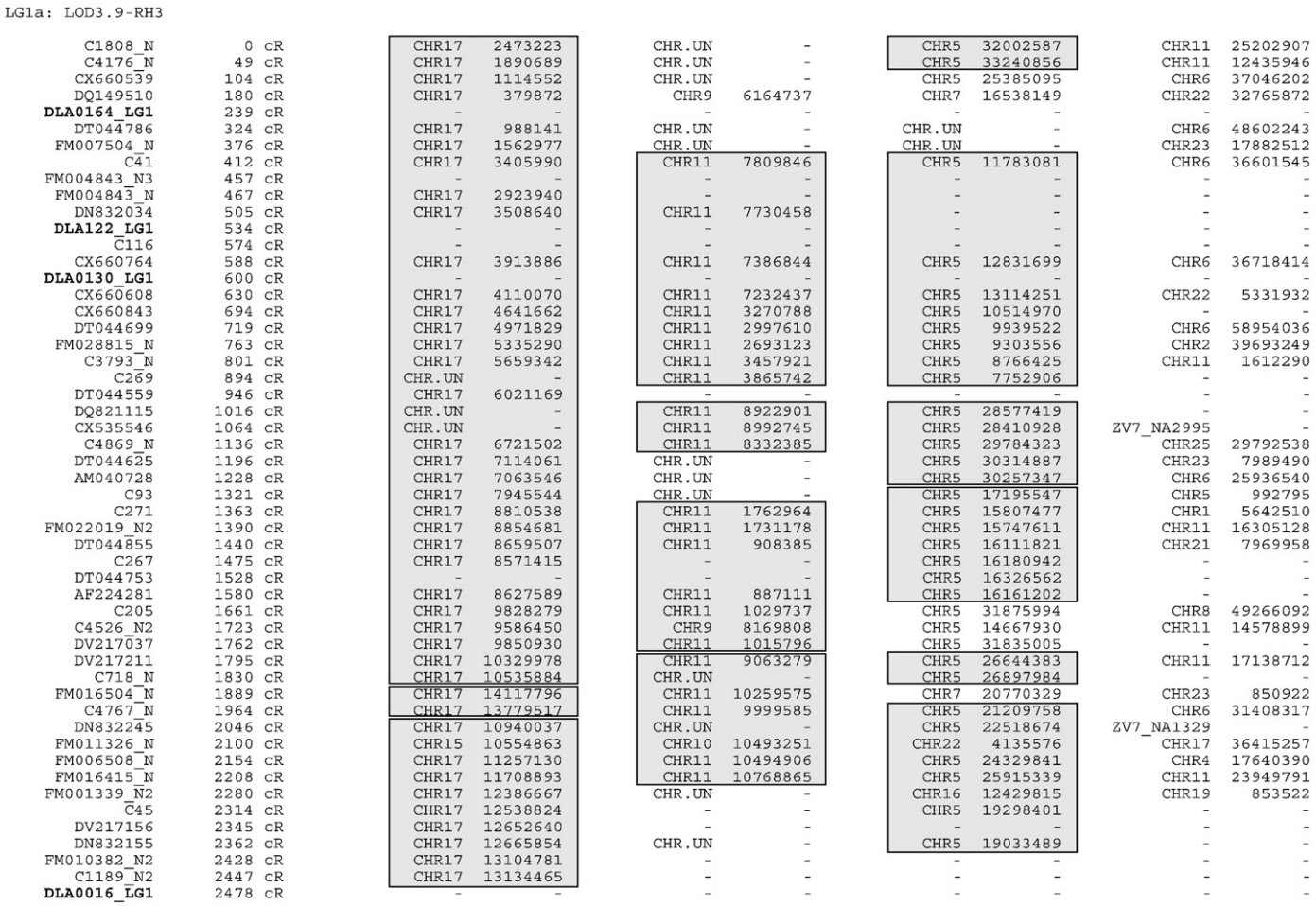
groups per chromosome. Chromosome RH maps were named after the linkage groups from the genetic maps [13,14]. Good correspondence was found between RH and linkage maps for most chromosomes. We found that linkage groups LG18 and LG21 were assigned to the same chromosome as that observed in the second generation linkage map. Linkage groups LG22 and LG25 were also found assigned to a unique chromosome. The main discrepancy between the genetic and RH maps concerned linkage group LG1 which we found was split in two chromosomes named LG1a and LG1b. One RH chromosome map contained no microsatellite from the genetic maps (LGx). The assigned RH groups represented 1222 markers (91% of the linked subset) and ranged in size from 49 to 2899 cR. Twenty-six of the RH groups containing three to 15 markers remained unassigned. These unassigned RH groups represented 120 markers (9% of the linked subset) and ranged in size from 13 to 723 cR. Given that the 91 RH groups represent 1342 markers out of a total of 1581, the probability to map a marker of interest in one of the existing RH groups can be estimated at ~85%. The RH map of sea bass chromosome LG1a is shown in Fig. 3. The whole RH map is available in Supplemental Fig. S1. Further cytogenetic studies would help confirm the assignation of the RH groups to the 24 chromosomes. Most of all, such studies would help standardise the sea bass karyotype.

Map construction has revealed an unexpectedly high resolution of our sea bass RH panel leading to a high number of RH groups (n = 91). By contrast, the seabream RH panel constructed at the same irradiation dose resulted in far fewer RH groups (n = 28) starting with less than 500 markers. Moreover the total size of the sea bass RH map amounts to 54,373 cR, which corresponds to 14 kb/cR (763 Mb/54,373 cR), compared to 140 kb/cR in the seabream RH map. This resolution power of the sea bass RH panel irradiated at 3000 rad may be a consequence of the cell type used in the panel construction. Maybe the generated DNA fragments were smaller than expected. As the sea bass RH map consists mostly of gene markers, some gaps between RH groups could be due to a weak marker density corresponding to gene-poor regions such as centromeres.

Stickleback orthologous genes corresponding to sea bass markers were identified based on the Ensembl gene annotation (Supplemental Table S2). Among the 1236 gene markers positioned on the map, 74 markers happened to correspond to 33 orthologous genes in the stickleback, i.e. a redundancy of two to five markers per gene. These 74 markers were designed from independent ESTs or contigs and therefore treated as distinct sequences after the CAP3 assembly step. For 29 out of the 33 genes, markers that tagged the same gene were co-localised or mapped to adjacent positions. Only in three cases were the two duplicates mapped to two separate positions. The blind mapping of these duplicate markers thus provides a quality control for map construction.

Comparative genomics

Synteny relationships were established from the gene markers mapped in the assigned RH groups and having an ortholog identified with location information in at least one of the model species. Out of 1083 markers with an orthologous relationship, 323 (30%) allowed anchorage between sea bass and the four model species, 347 (32%) with three of them, 245 (23%) with two and 168 (16%) with just one model species for a total of ~3000 anchors. Synteny relationships identified by two or more consecutive markers defined a conserved



LG1a: LOD3.9-RH3

C1808_N	0	cR	CHR17	2473223	CHR.UN	-	CHR5	32002587	CHR11	25202907
C4176_N	49	cR	CHR17	1890689	CHR.UN	-	CHR5	33240856	CHR11	12435946
CX660539	104	cR	CHR17	1114552	CHR.UN	-	CHR5	25385095	CHR6	37046202
DQ149510	180	cR	CHR17	379872	CHR9	6164737	CHR7	16538149	CHR22	32765872
DLA0164_LG1	239	cR	-	-	-	-	-	-	-	-
DT044786	324	cR	CHR17	988141	CHR.UN	-	CHR.UN	-	CHR6	48602243
FM007504_N	376	cR	CHR17	1562977	CHR.UN	-	CHR.UN	-	CHR23	17882512
C41	412	cR	CHR17	3405990	CHR11	7809846	CHR5	11783081	CHR6	36601545
FM004843_N3	457	cR	-	-	-	-	-	-	-	-
FM004843_N	467	cR	CHR17	2923940	-	-	-	-	-	-
DN832034	505	cR	CHR17	3508640	CHR11	7730458	-	-	-	-
DLA122_LG1	534	cR	-	-	-	-	-	-	-	-
C116	574	cR	-	-	-	-	-	-	-	-
CX660764	588	cR	CHR17	3913886	CHR11	7386844	CHR5	12831699	CHR6	36718414
DLA0130_LG1	600	cR	-	-	-	-	-	-	-	-
CX660608	630	cR	CHR17	4110070	CHR11	7232437	CHR5	13114251	CHR22	5331932
CX660843	694	cR	CHR17	4641662	CHR11	3270788	CHR5	10514970	-	-
DT044699	719	cR	CHR17	4971829	CHR11	2997610	CHR5	9939522	CHR6	58954036
FM028815_N	763	cR	CHR17	5335290	CHR11	2693123	CHR5	9303556	CHR2	39693249
C3793_N	801	cR	CHR17	5659342	CHR11	3457921	CHR5	8766425	CHR11	1612290
C269	894	cR	CHR.UN	-	CHR11	3865742	CHR5	7752906	-	-
DT044559	946	cR	CHR17	6021169	-	-	-	-	-	-
DQ821115	1016	cR	CHR.UN	-	CHR11	8922901	CHR5	28577419	-	-
CX535546	1064	cR	CHR.UN	-	CHR11	8992745	CHR5	28410928	ZV7_NA2995	-
C4869_N	1136	cR	CHR17	6721502	CHR11	8332385	CHR5	29784323	CHR25	29792538
DT044625	1196	cR	CHR17	7114061	CHR.UN	-	CHR5	30314887	CHR23	7989490
AM040728	1228	cR	CHR17	7063546	CHR.UN	-	CHR5	30257347	CHR6	25936540
C93	1321	cR	CHR17	7945544	CHR.UN	-	CHR5	17195547	CHR5	992795
C271	1363	cR	CHR17	8810538	CHR11	1762964	CHR5	15807477	CHR1	5642510
FM022019_N2	1390	cR	CHR17	8854681	CHR11	1731178	CHR5	15747611	CHR11	16305128
DT044855	1440	cR	CHR17	8659507	CHR11	908385	CHR5	16111821	CHR21	7969958
C267	1475	cR	CHR17	8571415	-	-	CHR5	16180942	-	-
DT044753	1528	cR	-	-	-	-	CHR5	16326562	-	-
AF224281	1580	cR	CHR17	8627589	CHR11	887111	CHR5	16161202	-	-
C205	1661	cR	CHR17	9828279	CHR11	1029737	CHR5	31875994	CHR8	49266092
C4526_N2	1723	cR	CHR17	9586450	CHR9	8169808	CHR5	14667930	CHR11	14578899
DV217037	1762	cR	CHR17	9850930	CHR11	1015796	CHR5	31835005	-	-
DV217211	1795	cR	CHR17	10329978	CHR11	9063279	CHR5	26644383	CHR11	17138712
C718_N	1830	cR	CHR17	10535884	CHR.UN	-	CHR5	26897984	-	-
FM016504_N	1889	cR	CHR17	14117796	CHR11	10259575	CHR7	20770329	CHR23	850922
C4767_N	1964	cR	CHR17	13779517	CHR11	9999585	CHR5	21209758	CHR6	31408317
DN832245	2046	cR	CHR17	10940037	CHR.UN	-	CHR5	22518674	ZV7_NA1329	-
FM011326_N	2100	cR	CHR15	10554863	CHR10	10493251	CHR22	4135576	CHR17	36415257
FM006508_N	2154	cR	CHR17	11257130	CHR11	10494906	CHR5	24329841	CHR4	17640390
FM016415_N	2208	cR	CHR17	11708893	CHR11	10768865	CHR5	25915339	CHR11	23949791
FM001339_N2	2280	cR	CHR17	12386667	CHR.UN	-	CHR16	12429815	CHR19	853522
C45	2314	cR	CHR17	12538824	-	-	CHR5	19298401	-	-
DV217156	2345	cR	CHR17	12652640	-	-	-	-	-	-
DN832155	2362	cR	CHR17	12665854	CHR.UN	-	CHR5	19033489	-	-
FM010382_N2	2428	cR	CHR17	13104781	-	-	-	-	-	-
C1189_N2	2447	cR	CHR17	13134465	-	-	-	-	-	-
DLA0016_LG1	2478	cR	-	-	-	-	-	-	-	-

Fig. 3. Radiation hybrid map of the sea bass chromosome LG1a. Marker names are listed in column 1. All names correspond to gene-based markers except names in bold that correspond to microsatellites. Column 2 represents cumulative distances expressed in centiRays (cR). Following columns correspond to comparative data with, from left to right, stickleback, pufferfish, medaka and zebrafish. For every marker, chromosome number and coordinates of the putative orthologs in the genome sequences of the four model species are displayed. Boxes represent CSO between sea bass and model species except zebrafish.

segment (CS) while a single marker identified a singleton. Oxford grids shown in Fig. 4 recapitulate the conserved segments (CS) between sea bass and stickleback (Fig. 4A), medaka (Fig. 4B) and pufferfish (Fig. 4C) identified from the assigned RH groups. The Oxford grid between sea bass and zebrafish (Fig. 4D) is shown only as a rough guide as zebrafish does not belong to the Percomorpha and is phylogenetically too distant from sea bass to establish a pattern of chromosomal events. Synteny relationships between sea bass and gilthead seabream were also indirectly investigated with the help of sea bass–medaka relationships established from this work and medaka–seabream relationships described by Sarropoulou et al. [18] (Fig. 4E).

Synteny relationships are best described with stickleback through 1014 anchors to the sea bass assigned RH groups. The RH map revealed 24 CS between these two species. Every sea bass chromosome consists of a unique CS with the stickleback while three stickleback chromosomes (GAC1, 4, 7) each consist of two CS with sea bass. This implied three inter-chromosomal rearrangements between these two species while 18 chromosomes remained without inter-chromosomal changes. Two inter-chromosomal rearrangements (GAC04-LG2/LGx and GAC07-LG3/LG14) were also observed by Kuhl et al. [12]. The third one (GAC01-LG13/LG24) was only observed by us. An additional break (GAC20-LG16/LG18) identified by a singleton in the RH map was observed by Kuhl et al. [12].

Synteny relationships between sea bass and medaka were established from 806 true anchors. The map revealed 24 CS between the two species. Every sea bass chromosome consists of a unique CS with medaka and conversely, which implies no synteny breakpoint between these two species. Synteny relationships between sea bass and pufferfish were established from 625 anchors revealing 26 CS between the two species. Of these 26 CS, 16 correspond to entire chromosomes with no major rearrangement. Four pufferfish chromosomes and one sea bass chromosome are composed of two CS implying five inter-chromosomal rearrangements since lineage split. The CS between sea bass LG3 and pufferfish TNI20 was identified from only two orthologous genes while most other pufferfish orthologs for this RH group were located in the "chromosome unknown". Moreover no other synteny was identified with TNI20 because of the assembly status for this chromosome. Finally, 13 chromosomes did not undergo any rearrangement since the evolutionary radiation of Percomorpha some 200 million years ago (MYA) [40,41]. These chromosomes are written in bold in Figs. 4A, B, C. The analysis of synteny relationships between sea bass and zebrafish revealed 47 CS. However many anchors between the sea bass and the zebrafish genomes were singletons putatively identifying additional CS. Due to the phylogenetic distance between the two species, genomes must have been intensively rearranged since lineage split, creating a high number of short CS which could possibly be identified with a higher density of orthologous markers.

On an intra-chromosomal scale, Conserved Segments Ordered (CSO) between two species are regions in which the order of orthologous genes is perfectly conserved due to the absence of

rearrangement after lineage split [42,43]. The identification of CSO allows to anticipate the localisation of all genes in a specific region given that the synteny has been established with the genome of a model species. Our sea bass RH map aimed at identifying these CSO with stickleback, medaka, pufferfish and zebrafish in order to benefit from the comprehensive sequencing of these genomes and to hypothesise the location on the sea bass genome of most if not all sea bass genes having a clear orthologous relationship with genes of

A

	GAC17	GAC05	GAC04	GAC08	GAC02	GAC19	GAC09	GAC11	GAC10	GAC03	GAC06	GAC15	GAC01	GAC07	GAC16	GAC20	GAC18	GAC21	GAC14	GAC13	GAC12	CHR.UN
LG1a	41											1										3
LG1b		36																				2
LG2			28																	1		
LG3														27								1
LG4				65								1	1						1			1
LG5			1		29																	2
LG6						40																
LG7					1		33															4
LG8		1						50				1							1	1	1	1
LG9							1		47				1									
LG10				1					1	55	1		1	1								
LG11									1		38											1
LG12												52		1			1					3
LG13													22									2
LG14													3	51								3
LG15		1											1		31							4
LG16										1						66						
LG17								2									35					
LG18-21													1			1		17				3
LG19			1				1	1											54			2
LG20												1								46		2
LG22-25						1		1													58	2
LG24													23		1			1				2
LGx			29							1												

B

	OLA05	OLA19	OLA10	OLA18	OLA04	OLA03	OLA06	OLA01	OLA08	OLA11	OLA17	OLA15	OLA22	OLA13	OLA14	OLA21	OLA16	OLA24	OLA20	OLA12	OLA09	OLA07	OLA02	OLA23	CHR.UN
LG1a	34												1				1					2			2
LG1b	1	26															1					1			5
LG2			20																		1				3
LG3				21							1														1
LG4					48			1												1					7
LG5						31																			2
LG6						1	29			1															6
LG7								24												1					5
LG8			1					1	42											1	1	2			1
LG9								1		28	1			1			1								6
LG10					2						48			1								1			4
LG11				1								28													1
LG12								1		3			41		1			2				1			2
LG13														18											1
LG14				2										3	33								1		6
LG15																20						1	1		7
LG16											1						48						1		6
LG17												1	1					30							
LG18-21							1												20				1		1
LG19								1	1											44	1				5
LG20													1								35				4
LG22-25					1				1													41		1	6
LG24																			1				14		7
LGx											1		1											22	1

C

	TNI11	TNI20	TNI01	TNI05	TNI13	TNI18	TNI03	TNI21	TNI15	TNI17	TNI10	TNI16	TNI07	TNI2	TNI08	TNI14	TNI06	TNI04	TNI12	TNI09	TNI19	CHR.UN
LG1a	23										1									2		12
LG1b							1		2					22						1		8
LG2			19																			5
LG3		2				1																19
LG4			35		1										1							20
LG5				19																		11
LG6					31																	5
LG7						24						1								1		9
LG8	1					3	18				1			2						1		17
LG9		1				1		19				1			1							16
LG10									35	1										1		15
LG11						1				21												7
LG12											45					1						6
LG13											4	14										4
LG14													36									9
LG15			1				1			1				26								
LG16								1	1						37							18
LG17							1									29						3
LG18-21							1										10					11
LG19							1											22				26
LG20											1							2	39			1
LG22-25					1															22	2	24
LG24							16															4
LGx					1				1											1	12	8

Fig. 4. Oxford grids between sea bass and (A) stickleback, (B) medaka, (C) pufferfish, (D) zebrafish, and (E) seabream. Lines correspond to sea bass chromosome maps named after the genetic map. Columns correspond to chromosomes of model fishes named as follows: DLA: sea bass chromosomes; GAC: stickleback chromosomes; OLA: medaka chromosomes; TNI: pufferfish chromosomes; DRE: zebrafish chromosomes; SAU: seabream chromosomes. The last column corresponds to the unlocalised part of the genome sequence (chromosome unknown). Conserved segments are figured in grey squares containing the number of orthologous markers that identified them. Singletons are figured in white squares. In the case of the sea bass–zebrafish Oxford grid, orthologous markers were figured indistinctly whether they consist of conserved segments or singletons because of the complexity of synteny relationships. In the case of the sea bass–seabream Oxford grid, conserved segments were deduced from the seabass–medaka and the seabream–medaka synteny relationships.

D

	DRE01	DRE02	DRE03	DRE04	DRE05	DRE06	DRE07	DRE08	DRE09	DRE10	DRE11	DRE12	DRE13	DRE14	DRE15	DRE16	DRE17	DRE18	DRE19	DRE20	DRE21	DRE22	DRE23	DRE24	DRE25	CHR.UN
LG1a	1	1		1	1	7		1			7						1		1		1	2	3		1	2
LG1b			2					1				13	1				1			2				2		2
LG2								1	1					14												3
LG3	2						6																			2
LG4		4				4		2		2	2					1	1			6	2	5				1
LG5	1				1	1	11										2	2							1	1
LG6		1		1			2										1	8							9	1
LG7	12					1								1					1		1	3				
LG8	1		26			1		1														1		1		3
LG9	1				1			1							2	2	1		13	1				1		2
LG10		20		1				1			1		1					2		2		1	1	2		
LG11												1	13				3		1	1						1
LG12		4				1							4				13		2	3	1			1		6
LG13	1				1										9	1										
LG14					4				1	6		1		4	3			2			9			2		
LG15	3					1			8		1					1						1				2
LG16	1	1														26			2	1						3
LG17			1									1	1				1			15			1			1
LG18-21		3				1																		14		3
LG19		1	1		7			4		7		1				1			1		8					4
LG20					9			10				1	1				1				4		1			1
LG22-25				1		3	1	7			1					1					1	23				2
LG24	3					2	2		1													2	1				1
LGx				11			1				1		1													

″E

	SAU21	SAU11	SAU22	SAU15	SAU2	SAU18	SAU12	SAU17	SAU24	SAU19-20	SAU6	SAU7	SAU8	SAU1	SAU9	SAU10	SAU14	SAU13	SAU5	SAU3	SAU16	SAU23	SAU25	SAU4
LG1a	2																							
LG1b		1																						
LG2			1																					
LG3				1																				
LG4																								
LG5						4																		
LG6							1																	
LG7								1																
LG8									1															
LG9										2														
LG10											5													
LG11												2												
LG12													1											
LG13														1										
LG14															1									
LG15																2								
LG16																	2							
LG17																		2						
LG18-21																								
LG19																								
LG20																					2			
LG22-25																						5		
LG24																								
LGx																								
	OLA05	OLA19	OLA10	OLA18	OLA04	OLA03	OLA06	OLA01	OLA08	OLA11	OLA17	OLA15	OLA22	OLA13	OLA14	OLA21	OLA16	OLA24	OLA20	OLA12	OLA09	OLA07	OLA02	OLA23

Fig. 4 (*continued*).

the model species. Moreover the comparison with several species allowed to simultaneously ascertain the extent and boundaries of shared CSO, reinforcing the probability of the hypothesis, while being able to reveal the break points that arose in some lineages. CSO with stickelback, medaka and pufferfish identified using the AutoGRAPH web server [32] are indicated in Table 2.

A total of 112 CSO were identified between sea bass and stickleback. Some chromosomes, such as LG3 and LG18–21, are remarkable; they did not undergo any intra-chromosomal rearrangement and consist of a perfect CSO. In contrast, chromosomes LG8, LG10, LG12 are the most rearranged. However the synteny relationships for these chromosomes are too complex to identify the rearrangement events that led to the synteny pattern. All other chromosomes underwent local insertions or inversions leading to three to seven CSO. By aligning both ends of 45,000 BAC clones on the stickleback sequence, Kuhl et al. [12] identified 139 rearrangements leading to a greater number of CSO than was found in the present study. Although the density of anchors between the two genomes is lower in our approach, its resolution is comparable and could be improved by mapping additional markers. The next step will consist in verifying CSO that differ between both studies.

A total of 92 CSO were counted between sea bass and medaka. AutoGRAPH detected one CSO in chromosomes LG2, LG18–21 and LG19 while chromosomes LG10 and LG12 were the most rearranged with eight CSO. All other chromosomes harboured two to six CSO. A total of 70 CSO were identified between sea bass and pufferfish. As a relatively large fraction of the genome sequence is unassembled, this value likely represents an underestimate and does not mean fewer recombination events in the pufferfish lineage compared to stickleback and medaka lineages. Hidden CSO may lie in the region conserved with the pufferfish “unknown chromosome” for instance in LG3, LG4, LG9 or LG10. The sea bass map provides elements for assembling the unknown set of genome sequences of pufferfish, such as contigs containing orthologs localised in these regions.

When comparing sea bass, stickleback, medaka and pufferfish with each other, particular regions are highlighted such as chromosomes LG18–21, LG3, LG19 and LG13 that underwent very few rearrangements since lineage split. This may reveal structural stability linked with evolutionary constraints for these chromosomes. Conversely chromosomes LG12, LG18 and LG20 were intensively rearranged. However in some cases the quality of the genome sequence assembly of the model species as well as a mapping analysis bias may be responsible for some heretical CSO.

Forty-one singletons were identified between sea bass and stickleback, 61 between sea bass and medaka and 45 between sea bass and pufferfish. These singletons will have to be investigated individually to establish if they are real or not. Indeed the orthologous location of a given sequence in a model species was defined as the highest hit on the genome of that species, but it may sometimes not correspond to the true ortholog.

Correspondence between the sea bass and seabream RH maps was established from their medaka synteny relationships and with the help of 37 orthologous markers spread over 19 chromosomes (Supplemental Table S3). No inter-chromosomal rearrangement was observed between the sea bass and the medaka nor between the seabream and the medaka [18], which implies no inter-chromosomal rearrangement between the sea bass and the seabream. Additional markers will have to be found to confirm the correspondence between

Table 2

Seabass	Stickleback		Medaka		Pufferfish		Zebrafish
	CS	CSO	CS	CSO	CS	CSO	CS
LG1a	1	3	1	6	1	4	2
LG1b	1	4	1	4	1	2	1
LG2	1	6	1	1	1	2	1
LG3	1	1	1	3	1	1	2
LG4	1	7	1	5	1	1	3
LG5	1	3	1	4	1	1	1
LG6	1	5	1	3	1	5	2
LG7	1	3	1	5	1	6	2
LG8	1	11	1	4	1	1	1
LG9	1	3	1	6	1	2	3
LG10	1	8	1	8	1	2	2
LG11	1	4	1	4	1	3	2
LG12	1	8	1	8	1	4	3
LG13	1	2	1	3	2	4	1
LG14	1	4	1	2	1	5	4
LG15	1	4	1	4	1	3	1
LG16	1	7	1	4	1	4	1
LG17	1	5	1	5	1	5	1
LG18–21	1	1	1	1	1	1	2
LG19	1	3	1	1	1	3	4
LG20	1	6	1	3	1	7	3
LG22–25	1	7	1	3	1	1	2
LG24	1	4	1	2	1	2	2
LGx	1	3	1	3	1	1	1
All chr	24	112	24	92	25	70	47

CS: Conserved segments.
CSO: Conserved segment ordered.

the five remaining chromosomes. A dense comparative map will also allow the identification of intra-chromosomal rearrangements between the two species.

Conclusion

For the first time a complete gene map of all 24 chromosomes of European sea bass is presented. Our RH map represents a new resource to facilitate genome navigation, to explore evolutionary history, to understand adaptation and to support marker-assisted selection of traits of aquaculture interest. The RH map complements the assembly of the draft resulting from the ongoing sequencing of the sea bass genome. Knowledge of all breakpoints and CSO with model species maximises the chances to map *in silico* any gene of interest knowing its location in the model genomes. Chromosomes that underwent few rearrangements during evolution have been identified and point at possible constraints to genome architecture. Based on these synteny relationships, the RH map should improve the assembly of sequenced model genomes. The ongoing efforts to establish synteny relationships with other Perciformes of aquaculture interest, such as the gilthead seabream and Nile tilapia, and fundamental models, such as the three-spined stickleback and haplochromids, will allow the quick transposition of research results to conspecifics. Access to functional information on genes and gene clusters in other taxa will directly benefit the interpretation of QTL in sea bass. Access to a dense genome map is essential as depth of coverage and length of reads often limit the assembly of next generation sequences. Therefore, our simplified method for the construction of a RH panel for a lower vertebrate from spleen is promising given the wealth of sequence data already appearing from next generation sequencing [44].

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ygeno.2010.07.007.

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