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First genetic linkage map for comparative mapping and QTL screening of brill (*Scophthalmus rhombus*)☆

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ABSTRACT

Genetic maps constitute valuable tools to detect genomic regions associated with complex traits and to go forward to understand their genetic basis. Flatfish include several species of great commercial value for which increasing genomic resources are available including genetic maps and EST databases. Application of comparative mapping strategies to flatfish is relevant to obtain genetic information associated with productive traits. The brill (*Scophthalmus rhombus*) is a flatfish species closely related to turbot (*S. maximus*) whose meat is highly appreciated in the Spanish market. The Junta de Andalucía local Government has begun a program to adapt this species to captivity for its future production. In this study, we developed the first genetic map of brill using the current turbot genetic map as starting point. This strategy enabled us to select a number of homogeneously distributed markers in the turbot map and to apply cross-species microsatellite amplification to obtain informative markers. Nearly two hundred microsatellites from the framework turbot map were used for validation, and 100 markers were finally informative for mapping. The parents and offspring of the two families (54 and 88, respectively) used to construct the genetic map were genotyped with this panel. All markers, except eleven, were successfully grouped and ordered in 24 linkage groups. Linkage groups and order of markers were highly consistent with the previous turbot genetic map. Linkage map information was used to carry out a preliminary study on growth-related QTL for body weight, length and Fulton's condition factor in the two families, as the main phenotypic traits of interest in this species.

1. Introduction

Linkage genetic maps constitute valuable tools for genomics studies in farm fish species, including identification of genomic regions associated with complex traits of interest and to go beyond by understanding their genetic basis (Canario et al., 2008; Danzmann and Gharbi, 2007). Furthermore, genetic maps provide the support to study genome organization, constitute the anchorage for analyzing genome evolution through comparative mapping and provide very useful landmarks for whole genome assembly (Kai et al., 2011; Naruse et al., 2009; Sanetra et al., 2009; Wang et al., 2011). To date, the highest whole genome and chromosomal information on fish is available at model species: zebra fish (*Danio rerio*), fugu (*Fugu rubripes*), Tetraodon (*Tetraodon nigroviridis*), medaka (*Oryzias latipes*) and stickleback (*Gasterosteus aculeatus*) (http://www.ensembl.org). This genome information has been applied for comparative mapping and gene mining strategies to identify candidate genes related to productive traits (Bouza et al., 2012; Loukovitis et al., 2011; Rodríguez-Ramilo et al., 2011, 2012).

Genetic maps have been developed in several important farmed fish species, such as Atlantic salmon (Gilbey et al., 2004), European seabass (Chistiakov et al., 2005), tilapia (Lee et al., 2005), gilthead sea bream (Franch et al., 2006), olive flounder (Kang et al., 2008), rainbow trout (Rexroad et al., 2008), channel catfish (Kucuktas et al., 2009), common carp (Cheng et al., 2009), grass carp (Xia et al., 2010), Japanese flounder (Castaño-Sánchez et al., 2010), Asian sea bass (Wang et al., 2011) and half-smooth tongue sole (Song et al., 2012). One of the most important applications of genetic maps is the identification of quantitative trait loci (QTL) for complex traits. This approach may eventually lead to the identification of particular genes underlying productive traits, which could be used for gene assisted selection (GAS) programs. Alternatively, trait associated markers could be used for marker assisted selection (MAS) programs, due to their correlation with the phenotypic trait variation.

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A main goal of genetic breeding programs in aquaculture is to increase growth rate, because it decreases rearing time thus increasing benefits. Nevertheless, the number of studies on growth related QTL in farmed fish is not large, mostly being focused in salmonids (Houston et al., 2008; McClelland and Naish, 2010; Moghadam et al., 2007; O'Malley et al., 2010; Sauvage et al., 2012), but also in species like tilapia (Cnaani et al., 2004), Asian sea bass (Wang et al., 2011) or European sea bass (Loukovitis et al., 2011) among others. Recently, several studies of QTL identification have been carried out in turbot for relevant aquaculture traits such as sex determination (Martínez et al., 2009), growth-related traits (Ruan et al., 2010; Sánchez-Molano et al., 2011), and disease resistance-related traits (Rodríguez-Ramilo et al., 2011, 2012). This has opened a new scenario to similar studies in related species and has increased the knowledge on the genetic basis of these traits in flatfish.

Flatfish (order Pleuronectiformes) are a relatively large group of teleost with ca. 570 species of worldwide distribution, comprising species of great commercial value. Several phylogenetic studies have been conducted using morphological and molecular data in this group which have established the genetic relationships between species and families (Azevedo et al., 2008; Chapleau, 1993; Hensley, 1997; Pardo et al., 2005). Application of new genomic tools such as comparative mapping using model fish genomes as a bridge is highly relevant to understand the genetic basis of production traits such as growth, resistance to pathologies or sex determination mechanisms. In addition, this approach could aid to understand the singular metamorphosis and reproductive genetic pathways of flatfish today not well known (Cerdà et al., 2010). Four genetic linkage maps have been reported in flatfish: Japanese flounder (Coimbra et al., 2003), Atlantic halibut (Reid et al., 2007), half-smooth tongue sole (Song et al., 2012), and turbot (Bouza et al., 2007, 2008, 2012), and another one is being finished in Senegalese sole (de la Herrán, pers. comm.). Comparative analysis and integration of flatfish genetic maps will bring a powerful tool for evolutionary research into Pleuronectiformes and will enhance the search of genome regions or candidate genes related to productive traits.

The brill (*Scophthalmus rhombus*) is a flatfish species closely related to turbot (*S. maximus*), distributed from 30° to 64° N along the coastal area at depths of 5–50 m (Vinagre et al., 2011). *S. rhombus* and *S. maximus* share similar habitat and life-history features, though they show a quite different diet and a little niche overlapping (Blanquer et al., 1992; Vinagre et al., 2011). It is a promising species for aquaculture in the Southern Atlantic–Mediterranean coast, since it is adapted to warm climate, shows high growth rates and its meat is highly appreciated in the Spanish market (Hachero-Cruzado et al., 2009). From 2002, the flatfish farming group at IFAPA Center "Agua del Pino" (Cartaya, Huelva, Spain), in collaboration with other Institutions, is focusing on the study of brill reproductive biology in captivity among other aspects, as a necessary prerequisite for the sustainable culture of this species (Hachero-Cruzado et al., 2007).

In this study, we developed the first genetic map in the brill (*S. rhombus*) using the existing turbot genetic map as starting point. This strategy enabled us to select a number of homogeneously distributed markers in the turbot map to obtain informative markers through cross-species microsatellite amplification, and to carry out a preliminary QTL screening on growth-related traits in the brill.

2. Materials and methods

2.1. Mapping families and DNA extraction

Two full-sib brill families of 54 and 88 offspring (Fam-1 and Fam-2, respectively) were obtained at IFAPA Agua del Pino experimental aquaculture station. Brill larvae were obtained from artificially fertilized eggs of a domesticated broodstock adapted to captivity. Larval culture method was the same as in Hachero-Cruzado et al. (2009). Brill families were kept under natural conditions of photoperiod in an open circulation system at 38–39‰ salinity, 5–8 ppm oxygen and 19–21 °C temperature. After the larval stage, fishes were fed *ad libitum* with dry pellets (R-2 Europa 22™® from Skretting) and programmed automatic feeders (T-Drum Feeder™, Arvotec), six times a day.

Fin tissue of analyzed individuals was cut and stored in 95% ethanol. Genomic DNA was extracted from the preserved fin samples using a standard phenol–chloroform protocol.

Body weight and length were recorded in both families to evaluate three growth-related traits: body weight, length and Fulton's condition factor (*FK*). This is a measure of fish fatness computed as $100 \times We/Le^3$, where *We* is the body weight of the fish (in grams) and *Le* is the length of the fish (in centimeters). The age of evaluation was one year post-hatching.

2.2. Microsatellite selection and genotyping

We took advantage of the high-resolution turbot map (Bouza et al., 2012) and the close relationship between brill and turbot (Pardo et al., 2005) to select and cross-amplify a number of homogeneously distributed markers in the turbot map following a sequential strategy. First, the panel of 98 homogeneously distributed turbot markers used for QTL identification (Martínez et al., 2009) was tested for cross amplification and polymorphism in a small set of brill individuals. Average distances between these markers were 18.4 cM and 13.8 cM according to the total and framework turbot genetic map lengths, respectively (Bouza et al., 2007, 2008), being below the minimum distance proposed for QTL detection (Dekkers and Hospital, 2002). The markers which showed positive amplification and polymorphism in brill, were genotyped in the parents of both brill families (Fam-1 and Fam-2) to check for informativeness and subsequent mapping. The distribution of informative markers was evaluated in the turbot map (Bouza et al., 2007, 2008) to look for the presence of gaps and genome coverage. Additional markers were then selected to fill gaps trying to have available marker every ~10 cM. We focused on the most polymorphic EST-linked markers available in turbot map due to their higher evolutionary conservation (Navajas-Pérez et al., 2012). This procedure was repeated twice and led to a total of 100 informative markers from 198 tested. PCR amplifications were carried out using the set of primers designed for turbot using reported technical conditions with slight modifications (Bouza et al., 2002, 2008; Navajas-Pérez et al., 2012; Pardo et al., 2007). Genotype data were obtained using an ABI 3730xl Genetic Analyzer and the GeneMapper 4.0 software (Applied Biosystems).

2.3. Linkage analysis

Linkage map was constructed with JoinMap 3.0 (Van Ooijen and Voorrips, 2001) using the Cross Pollinator (CP) model with unknown linkage phase. A chi-square test was carried out first in JoinMap to identify deviations from the expected Mendelian segregation patterns. Bonferroni correction was also considered for multiple tests (Rice, 1989).

Markers were assigned into different linkage groups with a critical LOD threshold > 3.0. The order of markers in each linkage group was established using LOD thresholds of 3.0 and maximum recombination thresholds of 0.4. In only a few cases less restrictive parameters were chosen. The linkage group assignment was confirmed with the *twopoint* option of Cri-Map (Green et al., 1990). Besides, marker orders within groups were also confirmed with the *all* option of this package. The Kosambi mapping function (Kosambi, 1944) was applied to estimate the genetic distances in centimorgans.

An integrated linkage analysis was performed to construct a consensus map with data from the two families using JoinMap. The average recombination frequencies (RF) and combined LOD scores were

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