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Age-dependent QTL affecting body weight in gilthead seabream (*Sparus aurata* L.)

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Abstract

We examined 24 maternal half-sib families of gilthead seabream to identify quantitative trait loci (QTL) associated with body weight at four time points during a production cycle. 57 brooders and 637 offspring were genotyped for 14 informative microsatellite markers, spanning linkage groups 1 and 21. The QTL detection method was based on half-sib interval mapping analysis through a linear regression approach. One QTL was found significant at all time points in linkage group 1, with its effect having different profile across time, and one QTL in linkage group 21 that seems to impact body weight at a later growth stage of the species. Current results verified previously published QTL for growth in the above linkage groups, using a different genetic background of seabream. These QTL can be considered as valuable candidates for use in marker-assisted selective breeding programs, aiming at high rates of genetic improvement for growth in *S. aurata*.

Keywords: Body weight, QTL, seabream, aquaculture, time point.

Introduction

Growth-related traits and morphometric characters are quantitative in nature, and are considered to be influenced by multiple genes across the genome, as well as from environmental factors. These traits can be improved by phenotypic selection, but the genetic gain can be markedly increased through marker-assisted selection (MAS) (Dekkers & Hospital, 2002; Andersson & Georges, 2004). Mapping of quantitative trait loci (QTL) explaining variation in the above traits is an important starting point to detect potentially useful markers. The potential of MAS to improve high-interest traits is increasingly evident in aquaculture species, including gilthead seabream. The steady increase of molecular tools (see also Loukovitis *et al*., 2011, 2012) has facilitated QTL mapping studies in this species. A series of genetic loci linked to body weight, morphology and sex have already been reported (Boulton *et al*., 2011; Loukovitis *et al*., 2011; Massault *et al*., 2011; Loukovitis *et al*., 2012, 2013) as well as QTL affecting disease resistance of fish to pasteurellosis (Massault *et al*., 2011). We have previously reported four QTL affecting body weight in *S. aurata*, using a commercial population from NIREUS S.A. aquaculture company (Loukovitis *et al*., 2011, 2012). Herein, we aim to verify the two most significant of the aforementioned QTL in a different genetic background (a commercial population from ANDROMEDA S.A. aquaculture company) as well as to examine their effects at different time points during the production cycle of this species.

Materials and Methods

We applied an interval mapping approach to search for QTL affecting body weight in 24 maternal half-sib families of gilthead seabream *Sparus aurata*. A total of 637 offspring, coming from a commercial broodstock of 57 brooders (33 males and 24 females) that were left to naturally mass spawn, were used. The collected eggs originated from a single spawning day, and rearing procedures followed the aquaculture company's common practice with the exception of avoiding size grading in order to preserve the whole spectrum of variance of the population. All seabream offspring were gradually anesthetized with 3-phenoxy-ethanol and each fish was PIT-tagged and fin-clipped, with the fin clip preserved in absolute ethanol for further DNA extraction. Measurement of body weight took place at four successive time points - 244, 447, 564 and 986 days post-hatch (DPH). During this procedure, all 637 progeny were gradually anesthetized and weighed to the nearest gram. A pedigree structure was obtained after parentage analysis with nine microsatellite markers (Loukovitis *et al*., 2011), and the size of the 24 families ranged from 9 to 87 progeny, with an average of 27.

Two linkage groups (LG1 and LG21) were chosen as candidates for harbouring growth-related QTL, using as a criterion the genome-wide significance of the detected QTL, as reported in Loukovitis *et al*. (2012). All brooders and offspring were genotyped for 14 informative microsatellite markers, covering the LG1 and LG21 groups, and a *de novo* linkage analysis was performed using CRI-MAP 3.0 software (Green *et al*., 1990). Given the pedigree structure obtained by parentage analysis, the QTL detection method was based on half-sib regression analysis as described by Knott *et al*. (1996), and was carried out using the GridQTL portal (Seaton *et al*., 2006). No fixed/random effects or covariates were fitted in the analysis. The 5% significance threshold values were determined at chromosome- and genome-wide levels, using permutation techniques (Churchill & Doerge, 1994) (1000 permutations). Furthermore, an estimation of the proportion of phenotypic variance explained (PVE) by the QTL was calculated according to the equation described by Knott *et al*. (1996). The detailed methods for genotyping, genetic linkage and QTL analyses are given in Loukovitis *et al*. (2011) and Loukovitis *et al*. (2012).

Results and Discussion

Linkage analysis confirmed mapping of the 14 microsatellites onto the linkage groups 1 and 21 (Fig. 1). The average marker interval per linkage group ranged from 2.1 (LG21) to 5.9 (LG1) cM (Kosambi centimorgan) with a mean across the two LGs of 4 cM. Mapping of markers was consistent with the respective LGs in Loukovitis *et al*. (2012) and in the second generation linkage map of the species (Tsigenopoulos *et al*., 2014), although their order and distance were not totally preserved. Such differences are expected for parameter estimates based on different populations and mapping panels.

Half-sib regression analysis in LG1 revealed a QTL for body weight that was significant (chromosome- or genome-wide) at all four time points. The QTL effect had a different profile (significance and variance) across time, with an increasing trend in genome-wide significance levels, reaching its maximum at 564 DPH and retaining chromosome-wide significance at 986 DPH (Table 1). At 564 DPH, the estimated proportion of phenotypic variance attributable to the QTL was quite high (16.2%), although this might be an overestimate due to the so-called Beavis effect (Xu, 2003). The body weight QTL was positioned at 23 cM at all time points, close to markers *CL2750Contig1* and *cDN14P0004O13*. We have previously reported a genome-wide significant QTL for body weight, located on the same region close to marker *cDN14P0004O13* (also referred as *VBC160*) on LG1 (Loukovitis *et al*., 2012). The verification of the specific QTL in another population, along with its constant effect across all developmental stages, indicates its robustness and good potential for its incorporation, through MAS, into seabream breeding programs. Preliminary results revealed that allelic substitution at the microsatellites *CL2750Contig1* and *cDN14P0004O13* had significant effects on body weight (data not presented). In addition, the QTL detected on LG1 showed its highest level of significance (genome-wide) at 564 DPH, where the 637 offspring had a mean body weight of 471.4 g (Table 1). The size of the progeny at this time point is quite close to seabream's harvest weight (350 - 450 g) for the fish market, which further highlights the commercial importance of this QTL.

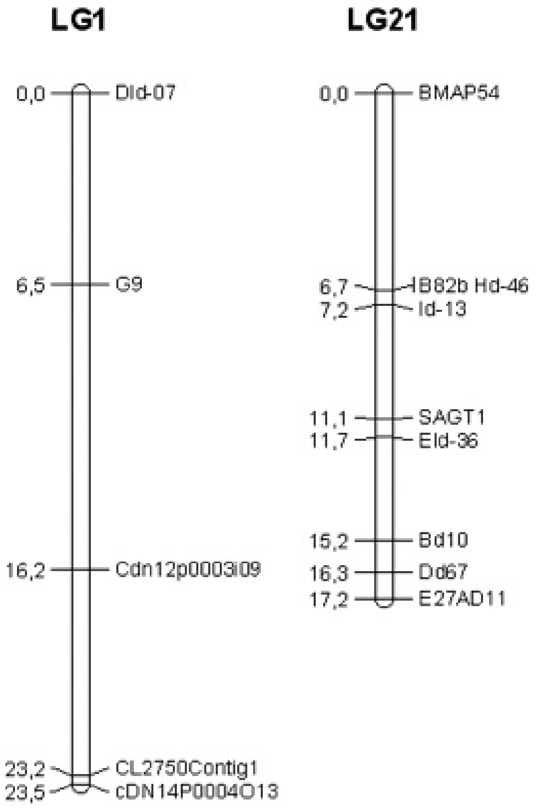

Fig. 1: *Sparus aurata* genetic linkage groups 1 and 21 (LG1, LG21) in Kosambi centimorgans (cM).

QTL analysis in LG21 detected a QTL for body weight that was significant (chromosome-wide) only at the fourth time point (986 DPH). The QTL was positioned at 17 cM, close to markers *Dd67* and *E27AD11*, having a PVE of 13.4% (Table 1). The *F*-ratios for the first three time points were far below the significance threshold, suggesting that this QTL impacts body weight at a later stage of development. A genome-wide significant QTL for body weight was also found in LG21 by Loukovitis *et al*. (2012). The progeny used in that study had a mean body weight of 691.7 g, which is higher compared to the mean body weights recorded at the first three time points of the present study (78.8 g, 238.9 g and 471.4 g, respectively). The difference in the QTL significance between the two studies (chromosome-wide *vs.* genome-wide) can be explained by the possible difference in allele frequencies at the QTL in different genetic backgrounds. Nevertheless, the results presented herein, along with those from Loukovitis *et al*. (2012), suggest

Table 1. Estimates of linkage group (LG) location (cM), significance (F-ratio) and proportion (%) of phenotypic variance explained (PVE) for body weight QTL in *Sparus aurata* through four different time points. Chromosome- and genome-wide significant QTL are indicated in bold. Mean body weight (g) of progeny in each time point is also given with standard error in parenthesis.

Trait (time point)	Mean body weight	Position	Closest marker(s)	F-ratio	5% significance threshold: Chromosome-wide	5% significance threshold: Genome-wide	PVE
			Linkage group 1 (LG1)				
1. **244 DPH**	78.8 (0.81)	23	*CL2750Contig1*, *cDN14P0004O13*	1.83	1.81	1.97	13.6
2. **447 DPH**	238.9 (2.25)	23	*CL2750Contig1*, *cDN14P0004O13*	1.92	1.85	2.01	14.6
3. **564 DPH**	471.4 (4.16)	23	*CL2750Contig1*, *cDN14P0004O13*	2.15	1.98	2.10	16.2
4. **986 DPH**	1085.8 (11.50)	23	*CL2750Contig1*, *cDN14P0004O13*	2.00	1.98	2.20	13.5
			Linkage group 21 (LG21)				
1. 244 DPH	78.8 (0.81)	-	-	1.08	1.85	1.97	-
2. 447 DPH	238.9 (2.25)	-	-	0.95	1.88	2.01	-
3. 564 DPH	471.4 (4.16)	-	-	0.71	1.94	2.10	-
4. **986 DPH**	1085.8 (11.50)	17	*Dd67*, *E27AD11*	1.92	1.90	2.20	13.4

DPH = days post-hatch, cM = Kosambi centimorgan.

that the phenotypic effect of the QTL on LG21 appears at a later stage of growth.

In conclusion, we verified the presence of previously published QTL for growth in a different genetic background of farmed gilthead seabream. One QTL was found to be significant at all time points in LG1. The experimental design allowed us to capture a developmental window along which the QTL's impact on body weight had a vertical parabolic shape with its directrix picking around 564 DPH. Another QTL was also found to be significant in LG21, with its effect popping up at a later growth stage of the species. To our knowledge, this study is the first to address a QTL analysis for body weight at different developmental stages of *Sparus aurata*. The nature of the above chromosomal regions associated with growth can be assessed more thoroughly by incorporating new types of markers, such as single nucleotide polymorphisms (SNPs), which can be readily obtained at low cost via next generation sequencing (NGS) methods. This type of approach is aimed at enabling fine mapping of the detected QTL, and shedding some light on the biological mechanisms behind the effect as well as at achieving high rates of growth, by means of MAS, in gilthead seabream.

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