Detection of QTL associated with three skeletal deformities in gilthead seabream (*Sparus aurata* L.): Lordosis, vertebral fusion and jaw abnormality

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**A B S T R A C T**

The presence of skeletal deformities in farmed fish is a major problem affecting aquaculture industry. In this study, the first QTL analysis for three of the most frequent deformities in gilthead seabream (*Sparus aurata* L.) is reported. A F1 full-sibling family consisting of 152 fish was genotyped by using a set of 13 multiplex PCRs (ReMsa1–ReMsa13). This PCR includes 106 microsatellite markers from the genetic map of this species. For vertebral fusion, one significant QTL (located in linkage group LG 21) and two suggestive QTL (located in LG4 and LG13, respectively) were detected. For lordosis, one significant QTL (located in LG9) at chromosome and genome-wide level and one suggestive QTL (located in LG22) were detected. For jaw deformity, one significant QTL (located in LG13) and one suggestive QTL (located in LG16) were detected. The percentages of variance explained by the QTL effect ranged from 1.6 to 11.4%. Cld-26-H and Cld-03-F were the two closest-to-jaw-deformity-significant-QTL microsatellite markers and showed a statistical association between male allelic segregation and phenotype. Hd-46-T was the closest-to-vertebral-fusion-significant-QTL microsatellite marker and showed a statistical association between male allelic segregation and phenotype. Considering these results can help to decrease the prevalence of these deformities in future by Marker Assisted Selection breeding and to solve one of the most important aquaculture industry problems. However, additional analyses in other gilthead seabream families are needed in order to confirm these QTL.

**Statement of relevance**

The results of the present study show the first QTL identified for skeletal deformities in gilthead seabream, which could help to decrease these deformity prevalence. These results, although in preliminary way, could help to solve one of the most important aquaculture industry problems.

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1. **Introduction**

The presence of morphological abnormalities in farmed fish is currently a major problem in aquaculture as it entails significant economic losses (Boglione and Costa, 2011). Gilthead seabream (*Sparus aurata* L.) is one of the marine species of Mediterranean aquaculture with the highest production; its annual production reached 179,924 tons in 2013 (APROMAR, 2014). And its production is also affected by deformities (Boglione and Costa, 2011; Prestinicola et al., 2013). This species is commercialized as whole fish, so deformed fish affect negatively to the turnover of hatcheries and on-growing companies, as their presence downgrades the quality of the product, thus, reducing its value. That is the reason by which deformed fish elimination prior to batch commercialization must be performed, significantly increasing the production costs.

Skeletal deformities, such as those affecting neurocranium or head, vertebral column and appendicular skeleton, are the most significant deformities since they affect directly to production traits. Head deformities include those affecting jaw and opercular complex. Jaw abnormalities include upper or lower jaw torsion, and upper or lower jaw extension in different magnitude, and have been associated with lethal effects (Afonso and Roo, 2007). Moreover, customers rarely accept fish showing jaw malformations (Bardon et al., 2009). Lordosis, scoliosis, kyphosis and vertebral fusion are the most frequent vertebral column deformities (reviewed by Boglione et al., 2013) and affect fish appearance, but, in addition, they also lead to physiological alterations that result in a decrease of fish commercial traits value: a lower growth rate, a higher mortality during handling and an increased difficulty of filleting. Furthermore, the effect of these deformities in animal welfare must be also considered (Gjerde et al., 2005; Karahan et al., 2013).
It has been demonstrated that controlling many environmental parameters such as biotic and abiotic, physiological, xenobiotic, nutritional and rearing factors, the prevalence of these deformities can be minimized, but not eliminated (Bardon et al., 2009). So, animal breeding control could be a good strategy to continuously and permanently decrease the prevalence of these deformities, in case they are also determined by a genetic factor. In the context of PROGENSA® (a Spanish Breeding Program), heritability from 0.06 to 0.11 for lack of operculum breeding program), heritability from 0.06 to 0.11 for lack of operculum and LSK complex (lordosis–scoliosis–kyphosis) under different breeding conditions, phenotype of breeders and offspring handling were found (Negrín-Báez et al., 2015). Moreover, this study provided really useful populations to search for quantitative trait loci (QTL) that were related to these deformities. A QTL is a genomic region closely linked to major effects genes that show association with phenotypic variations. The variation in quantitative traits may be controlled by those larger effects genes beside the interaction of a large number of minor effect genes (Geldermann, 1975). Moreover, QTL may provide DNA markers tightly linked to traits-affecting genes. Such markers can be used in Marker-Assisted Selection (MAS) within a breeding program. The advantages of considering MAS are notable as compared with the traditional selective breeding. As having an accurate linkage map is crucial to search for QTL, since the density of genetic markers is an important factor to detect them (Rodríguez-Ramilo et al., 2014). In gilthead seabream, a linkage map based on 204 microsatellite markers and 26 linkage groups (LG) is available (Franc et al., 2006). Moreover, a set of 13 multiplex PCR assays formed by 106 specific microsatellite markers that cover all LG in the genetic map of this species is also available (Negrín-Báez et al., 2014). Multiplex PCR is a highly effective tool to reduce cost per reaction and to minimize genotyping errors during the sample analysis process (Navarro et al., 2008).

In gilthead seabream, QTL for traits of commercial interest have been detected by using microsatellite markers: several QTL related with growth, sex determination and several morphometric traits were found by Loukoutis et al. (2011, 2012, 2013), two QTL for resistance to Pasteurellosis were found by Massault et al. (2010) and one significant QTL for morphometric traits and two suggestive QTL for stress response to confinement were detected by Boulton et al. (2011). However, no QTL for frequent vertebral column deformities have been reported. So, the main objective of the present study was to analyze and scan for QTL affecting lordosis, vertebral fusion and jaw abnormalities in a family of gilthead seabream, being a first approximation to the genetic determination of these deformities.

2. Material and methods

2.1. Fish, trait measurement and parental assignment

Fish were obtained from a directed mating established with two deformed breeders (lordosis). These breeders were descendants (F1) of two deformed sires and one normal dam, this mating is described in detail by Negrín-Báez et al. (2015). Fish were cultured at the industrial-like facilities of the Marine Science and Technology Park of the University of Las Palmas de Gran Canaria (PCTM-ULPGC, Gran Canaria, Spain) under intensive rearing conditions, described by Roo et al. (2009). The rearing was carried out in 500 l fiberglass tanks with commercial feed provided by automatic feeders. Water flow was 0.5 l min⁻¹, dissolved oxygen concentration was 5.9 ± 0.1 ppm and water temperature ranged from 19.3 ± 0.1 °C at the beginning of the experiment to 23 ± 0.1 °C at the end of the experiment. At 120 days post-hatching (3.74 ± 1.24 g) (mean ± standard error), fish were slaughtered and an initial visual phenotypic analysis was carried out in order to determine the presence of any type of deformity. Three types of deformities were observed: vertebral fusion, lordosis and jaw abnormality. A sample of 152 offspring was selected for QTL mapping and the initially detected deformities were re-analyzed by soft X-ray monitoring (Model Senographer-DHR, General Electric, USA). In fish suffering from vertebral fusion or lordosis, after identifying the vertebra where the deformity was produced, its position was located by counting vertebrae in anterior–posterior direction.

2.2. Genotyping

Offspring and their parents were genotyped by using 13 multiplex PCRs (ReMso1 to ReMso13), previously described by Negrín-Báez et al. (2014). These multiplex PCRs include a total of 106 microsatellite markers located on the genetic map of this species (Franch et al., 2006; Senger et al., 2006) and cover the 100% of the LG. The average distance between these microsatellite markers was 13.5 cm.

2.3. QTL mapping

A linear full-sibling regression analysis (Knott et al., 1996) was used to detect QTL for each deformity. Phenotypes were coded as normal (1) or deformed (2). The analysis was performed by using the GridQTL software (http://www.gridqtl.org.uk) (Seaton et al., 2006). Chromosome-wide and genome-wide significance thresholds were estimated by implementing a bootstrapping method at P = 0.05 and at P = 0.01 (Churchill and Doerge, 1994). According to chromosome-wide level, QTL was considered suggestive when its significance was between 5% and 1% and significant when it was below 1%. According to genome-wide level, QTL was considered significant when its significance was between 5% and 1% (Rodríguez-Ramilo et al., 2011). No fixed factor or covariate was included in the model. Confidence intervals were calculated by bootstrapping the samples 10,000 times (Visscher et al., 1996).

2.4. Genotypic association analysis

Contingency tables and Pearson chi-square tests were carried out in order to determine the association between phenotype (normal vs deformed) and allele segregated of microsatellite markers that were close to each QTL. To get that, alleles were codified to discriminate between those coming from the sire (s1 and s2) and those coming from the dam (d1 and d2). The allelic segregation was tested separately (from the sire and from the dam) for each microsatellite marker by using the statistical software package SPSS (PASW Statistics v18). Significant association was considered when P ≤ 0.05.

The QTL effect in terms of association between phenotype and genotypes of microsatellite markers that were close to each QTL was also determined by using the statistical software package SPSS (PASW Statistics v18). A one-way analysis of variance (ANOVA) was performed on the phenotypic values of each offspring and individual genotypes (s1d1, s1d2, s2d1, s2d2) were used as fixed factor. Each ANOVA provided a corrected Rsquared value that represented the reduction of the overall phenotypic variance of traits due to the model fitting, thus, providing the proportion of the trait variance predictable for each genotype.

3. Results

3.1. Phenotypic data

After X-ray analysis of the 152 offspring, 20 fish showed vertebral fusion, 42 fish showed lordosis, 13 fish showed jaw deformity and 77 fish showed no deformity. For vertebral fusion, the position of the affected vertebrae ranged from 6 to 10, the mean was 7.7 ± 1.0 and the most frequent ones were 7 and 8. For lordosis, the position of the affected vertebrae ranged from 6 to 20, the mean was 9.7 ± 2.0 and the most frequent one was 9. All jaw abnormalities found were of
pugheadness type; this deformity is produced when the ethmoid region and upper jaws are anteroposteriorly compressed.

3.2. Genotyping

106 microsatellite markers were genotyped. 93 out of them were informative and the total map length covered by them was 748.5 cM. These 93 microsatellites represented in the genetic map are shown in Fig. 1. The average distance between these microsatellite markers was 14.6 cM and the average number of microsatellite markers per linkage group was 3.9.

3.3. QTL mapping

Seven QTL were reported in this study. Their linkage groups, positions and confidence intervals are shown in Table 1 and their locations on the genetic map are shown in Fig. 1. For vertebral fusion, one significant QTL (QTLFV3) and two suggestive QTL (QTLFV1 and QTLFV2) were detected. For lordosis, one significant QTL (QTLOR1) at chromosome and genome-wide level and one suggestive QTL (QTLOR2) were detected. For jaw deformity, one significant QTL (QTLJW1) and one suggestive QTL (QTLJW2) were detected.

3.4. Genotypic association analysis

The close microsatellite markers, their allelic significant association for breeders and percentage of variance phenotypic explained by genotype (R²) for each QTL are shown in Table 1. The Ed-31-T (7.3 cM) marker was analyzed for QTLFV1 and Bd-29-H (17.3 cM) marker was analyzed for QTLFV2. Two closely located microsatellite markers were analyzed for QTLFV3, Hd-46-T and Dld-12-F (0 cM), however, Dld-12-F was an uninformative marker, as breeders were homozygous for this locus. For QTLOR1, closely located microsatellite markers Gd-78-F (40.5 cM) and Cld-89-H (26.7 cM) were also uninformative (breeders were also homozygous for this locus), so the marker Dld-14-H was analyzed. For QTLOR2, close markers analyzed were Bd-61-H and Dld-24-T (0 cM). The closest-to-QTLJW1 and QTLJW2 markers Cld-26-H (27.9 cM) and Cld-03-F (30 cM); and Cld-21-F (18.2 cM) were informative, respectively.

4. Discussion

The vertebral column of gilthead seabream has 24 vertebrae distributed in four regions in anterior–posterior direction: cranial, pre-haemal, haemal and caudal (Boglione et al., 2001). Chatain (1994) described lordosis in gilthead seabream fingerlings and studied the incidence according to the position of the affected vertebrae, and described that 50% of lordosis was between the 8th and 11th vertebrae, being the vertebra 9th the most frequent one. Vertebral fusion affects mainly haemal and caudal regions, but they have been observed all along the vertebral column. Pugheadness, cross-bite and lower jaw reduction or elongation are the main types of jaw abnormalities that can affect Mediterranean-aquaculture reared fish, but data from both experimental and reared fish demonstrate that pugheadness is the most frequent jaw abnormality in gilthead seabream (Boglione et al., 2013). All these data are similar to the phenotypic results observed in the present study.

Microsatellite markers are useful molecular markers for medium-high density maps. In the present study, genotyping was conducted by using 106 microsatellite markers from 13 multiplex PCRs and located in the linkage map for this species (Franch et al., 2006; Senger et al., 2006). The average distance between these microsatellite markers was 14.6 cM. This distance is shorter than the recommended maximum distance at QTL searching (20 cM) (Massault et al., 2008), but larger than in other QTL searching studies for this species (Boulton et al., 2011; Loukovitis et al., 2011, 2012, 2013). With regard to the latter, it must be noted that, in the present study, 60.3% (748.5 cM) of total length of the genetic linkage map (1241.9 cM) has been covered; while in the other studies, the covered length was significantly lower: 495.4 cM by Loukovitis et al. (2012) and 472.0 cM by Boulton et al. (2011).

In aquaculture, QTL mapping studies that are based on detection within families have been demonstrated to be successful methods for most marine fish species, since they are prolific and it is easy to obtain large families. In this study, the analyzed family size was no large (154 fish); however, the methodology to detect QTL was based on linear

![Fig. 1. Genetic linkage map of gilthead seabream with 93 microsatellite markers analyzed. QTL localization for lordosis, vertebral fusion and jaw deformity detected in a F1 full-sibling family are presented.](image-url)
regression by using the GridQTL software, which has been evidenced to be robust for use with discrete characters, including binary traits (Gorman et al., 2011), such as deformities.

The present study identifies, for the first time, seven QTL for three of the most relevant and frequent skeletal deformities in gilthead seabream, what support the genetic component for this trait recently proposed by Lee-Montero et al. (2014) and Negrín-Báez et al. (2015). For vertebral fusion, one of the three QTL detected in this study was considered significant (QTLFV3), it was located in LG21. This is a short LG, so association between markers and detected QTL should be more powerful (Massault et al., 2010). Indeed, its closest analyzed microsatellite marker (Hd-46-T) showed a significant association between male’s allelic segregation and presence of the deformity in offspring, additionally, its genotype explained the 36% of the phenotypic variance. One of the two QTL found for lordosis (QTLOR1) was considered significant, it was located in LG8. Remarkably, this QTL was considered significant also at genome level, which is a more stringent statistical level (Loukovitis et al., 2012). However, no significant association was detected between the marker genotypes of its LG and presence of the deformity. This QTL as well as the one for vertebral fusion, confirm the genetic origin of these spinal deformities. Moreover, these two QTL are located in the same LG (LG9 and 21) that significant QTL reported for growth (Loukovitis et al., 2012), morphology (Loukovitis et al., 2013) and size traits (Bouton et al., 2011) for this specie. This, together with the high and positive genetic correlations found by Lee-Montero et al. (2014) between characters of growth and column deformity, support the location of these QTL of this study.

Jaw deformity is also a frequent skeletal deformity, although it entails a lower economic impact. Many studies have related its prevalence with environmental conditions (Boglione et al., 2013), but not with genetic factors. Indeed, Lee-Montero et al. (2014) estimated a close-to-zero heritability for head deformities, but it can be explained because, beside jaw deformities, they included other head anomalies that are more difficult to evaluate and that could increase the environmental variance. A significant QTL for this deformity was located in LG13 (QTLJW1), as well as a suggestive one in LG16. A significant association between female’s allelic segregation and presence of the deformity was detected in two close microsatellite markers (Cld-26-H and Cld-03-F). More concretely, these markers genotypes explained the 11.4% and 7.8% of phenotypic variance, respectively. This suggests that this QTL could be located near the analyzed markers. Indeed, these markers are separated 2.1 pb according to the genetic map (Franch et al., 2006). Considering all of the above, the detection of this QTL evidences, for the first time in gilthead seabream, that jaw deformity has also a genetic determination.

On the other hand, it must be considered that the three significant QTL of this study were detected by using a unique full-sibling family. And that this method has some limitations: the found QTL only segregate in two breeders and they could not be representative for other gilthead seabream families (Ruan et al., 2010; Wang et al., 2006).

5. Conclusions

The results of the present study show the first QTL identified for lordosis, vertebral fusion and jaw deformity in gilthead seabream, which could help to decrease these deformities prevalence in future by MAS-implemented breeding programs increasing the numbers of analyzed molecular markers and confirming it in other gilthead seabream families. These results, although in preliminary way, could help to solve one of the most important aquaculture industry problems.

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References


Table 1

<table>
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<tr>
<th>QTL</th>
<th>LG Position (CI)</th>
<th>F</th>
<th>% CW</th>
<th>% CW Marker</th>
<th>X2 (σ2)</th>
<th>X2 (Q2)</th>
<th>R2</th>
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<td>8 (8–16)</td>
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<td>6.3</td>
<td>17</td>
<td>3.9</td>
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<tr>
<td>QTLFV2</td>
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<td>12 (7–12)</td>
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<td>6</td>
<td>14.9</td>
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<td>1.3</td>
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<tr>
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<td>16.4</td>
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Bold values indicate significance at P ≤ 0.05.


