EVIDENCE OF A PLEIOTROPIC QTL INFLUENCING COMPONENTS OF EARLY DEVELOPMENT IN DOUBLE HAPLOID LINES OF RAINBOW TROUT

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INTRODUCTION

Due to the great biological flexibility of many fish species, different designs that are not available in farm animals can be carried out using chromosomal manipulations. Completely homozygous clonal lines can be accomplished by successive rounds of androgenesis. These clonal lines can be crossed to obtain F1 parents and a double haploid mapping population can then be obtained using another round of androgenesis. Added power for QTL detection can be obtained from using clonal lines that differ widely for the trait under consideration. A rainbow trout data set on traits affecting early development was developed with such a mapping population, and analysed using composite interval mapping. This suggested that QTL affecting early development rate were segregating in some of the linkage groups of the AFLP marker map (Robison et al., 2001). In the present paper, our main interest is to test the hypothesis that some of the QTL identified were pleiotropic in their effect upon important traits in early development. This was done in a multi-step procedure: (i) confirming regions that show evidence of QTL using step-wise single-trait regression; (ii) extending this analysis using a Bayesian multi-locus single-trait method to estimate the number of QTL segregating in each linkage group and to dissect putative QTL positions with high resolution based on the posterior QTL intensity; (iii) identifying from these results, chromosomal regions that are indicated to hold single QTL affecting more than a single trait; (iii) testing the hypothesis of a simple linkage versus a single pleiotropic QTL using a multiple-trait least squares framework.

MATERIALS AND METHODS

Populations. The double haploid mapping population was developed from an all male (XY) F1 population obtained using crosses of all males (YY-SW) and all female (XX-OSU) clonal lines that show divergent hatching times in the laboratory. A sample of the F1 individuals were induced to spawn prior to 1 year of age using intraperitoneal injection of pituitary acetone extract three times per week. The sperm of the F1 individuals was used to obtain 2 groups of androgenetic rainbow trout (n = 206). Successful androgenesis from an F1 parent resulted in diploid organisms that contain two sets of identical paternal chromosomes with an equal proportion of male and female individuals. Two traits related to early development were examined; accumulated temperature units at hatching (ATH; mean 705.6, SD 76.82), and length at swim up stage (LEN; mean 63.66 mm, SD 19.68). The marker map contained 12
linkage groups, spanning 974.6 cM (about 40% of the rainbow trait genome), obtained from segregation of 136 AFLP markers. The mean distance between markers in the linkage groups was about 8 cM, with 10 cM standard deviation. A more detailed outline of the experimental set-up is presented by Robison et al. (2001), and power-related issues regarding double haploid individuals were discussed by Martinez et al. (2002).

**STATISTICAL ANALYSIS.**

**Single-trait step-wise regression.** A preliminary statistical analysis of the data set revealed that the slight increase in environmental temperature between the groups produced a significant effect in all traits considered. Due to this finding, the QTL analysis was carried out using the phenotypes corrected for environment. To control genetic background, marker cofactors were selected using stepwise regression and, except for those on the chromosome under investigation, included as covariates in subsequent analyses.

**Single-trait Bayesian analysis.** Parameter estimation in the Bayesian model was conducted using Metropolis-Hastings-Green (MHG) algorithm (Sillanpää and Arjas, 1998) to provide posterior samples of unknowns given the data and the prior distribution. The conditional distribution of the vector of unknowns given the observed data can be expressed in the form of a product of a likelihood and the prior information (Sillanpää and Arjas, 1998):

\[
p(\theta \mid y, G, X_\circ, m) = \frac{p(G \mid m)p(X_\circ \mid \theta, m, G)p(y \mid \theta, m, G, X_\circ)p(\theta \mid m)}{p(y, G, X_\circ \mid m)}
\]

where \(\theta\) is the vector of the unknown parameters, such as the number of QTL in the linkage group \(N_{qtl}\), the location of the QTL \(l\), the genotypes at each location, the regression parameters in the likelihood, the genotype of the background effects and missing genotypes. Moreover, \(G\) is the observed marker genotype in the linkage group under analysis, \(X_\circ\) is the observed marker information in other chromosomes, \(y\) is the phenotypic data and \(m\) is marker map providing information on the recombination fraction between markers. All priors were assumed to be uniform (within a reasonable range), except the number of QTL was assumed to be truncated Poisson with mean 2 (before truncation) and maximum 3. The range of proposal distributions were specified after several preliminary test runs in each of the linkage groups analysed. For the final analysis, proposals giving adequate mixing of the chains were used to run a single long chain \((3 \times 10^6)\). Results are presented in terms of the distribution of the number of QTL, QTL intensities and the mean of the distribution of additive effects within an interval of 1 cM, where the mode of the QTL intensity was located in the linkage group.

**Multiple trait regression.** The multiple trait analysis was carried out using least squares, regressing the phenotypes on conditional probabilities of QTL genotypes along the linkage group. A sequential testing procedure was carried out to determine the underlying genetic model (Knott and Haley, 2000). In particular we were interested in whether a model with two linked QTL, one affecting each trait, fitted the data better than one with a single pleiotropic QTL affecting both traits. An approximate likelihood ratio test was used and the significance level determined using the parametric bootstrap.
RESULTS
Screening for possible pleiotropic QTL from single-trait analyses. The posterior distribution of the number of QTL in the linkage groups and the corresponding posterior expectation \(E(N_{\text{qtl}} \mid \text{Data})\) was used to quantify segregation of QTL. According to this criteria, there was strong evidence for a QTL segregating for ATH and a QTL segregating for LEN in LG-12 where the posterior probability mass concentrated at \(N_{\text{qtl}} = 1\). The posterior expectation was equal to 1 in both cases. In LG-9 there was strong evidence of segregation for a QTL affecting ATH, but there was no evidence for any QTL segregating for LEN. There is little evidence of segregation of QTL in other linkage groups (see table 1). Therefore all further analyses presented in this paper concern LG-12 only.

Table 1. Posterior probability of number of QTL and the corresponding posterior expectation in different linkage groups that show putative evidence of QTL in traits ATH and LEN

<table>
<thead>
<tr>
<th>NQTL</th>
<th>ATH</th>
<th>LEN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LG-5</td>
<td>LG-6</td>
</tr>
<tr>
<td>0</td>
<td>0.96</td>
<td>0.86</td>
</tr>
<tr>
<td>1</td>
<td>0.04</td>
<td>0.11</td>
</tr>
<tr>
<td>2</td>
<td>0.00</td>
<td>0.02</td>
</tr>
<tr>
<td>3</td>
<td>0.00</td>
<td>0.01</td>
</tr>
</tbody>
</table>

\(E(N_{\text{qtl}} \mid \text{data})\)

|       | 0.04 | 0.18 | 0.78 | 0.24 | 1.01 | 0.45 | 0.15 | 0.04 | 0.33 | 1.09 |

Figure 1. QTL intensity of traits ATH and LEN in linkage group L-12 and LRT for least squares analysis. In the figure BAYES is the posterior QTL intensity for ATH and LEN (left axis), STLRT is the approximate likelihood ratio test of a single versus no QTL evaluated at every cM along the linkage group for ATH and LEN (single trait analysis) and MTLRT-PLE is the test statistic of a pleiotropic QTL influencing both traits versus no QTL (right axis). Ticks in the X axis denotes markers positions.
Location of putative QTL on LG12 from single-trait analyses. The posterior QTL intensity was used to locate QTL in the linkage groups that show putative QTL activity. The mode of the QTL intensity for ATH and LEN was 12.2 and 15 cM, respectively (figure 1). The mean of the distribution of additive effects of the QTL, had opposite effects, but similar in magnitude (-0.328 SD and 0.362 for ATH and LEN, respectively). Inspection of the posterior distributions for location suggested that the effects could arise from a single pleiotropic QTL, and this was tested below (see figure 1).

Multiple-trait regression analysis. The multiple-trait regression analysis initially fitted single-trait models on LG-12 (see figure 1 for the profile likelihood). These single-trait analyses were consistent with the Bayesian analysis giving evidence of QTL at positions 12 and 15 cM for ATH and LEN, respectively (figure 1). Within the multiple-trait framework, fitting a pleiotropic QTL affecting both traits was significantly better than no QTL. The best location was at 14 cM and the effect on each trait was significant. Comparing this model with the likelihood of a model with two separate QTL, each affecting one trait (at the best locations obtained from for the single trait analyses) gave an approximate likelihood ratio of 2.81 which was lower than the 5 % significance threshold (4.78) obtained from the parametric bootstrap for this test. From this analysis it is possible to conclude that there is no evidence to postulate two distinct QTL and hence the null hypothesis of a single pleiotropic QTL affecting both ATH and LEN is sufficient to explain the data.

CONCLUSION
The results have provided evidence for a single QTL segregating in LG12 that has pleiotropic effects on ATH and LEN, traits that are of importance not only on early development of the rainbow trout, but also due to their relationship with sexual maturity as has been observed in natural populations. The Bayesian analysis provided strong evidence of a single QTL for each trait segregating on LG12, and the multiple-trait analysis has shown that these findings are satisfactorily explained by a single pleiotropic QTL affecting both traits. The mean of the additive effects of the QTL conditional on the most likely interval, where the putative QTL lie in the linkage group, is about one third of the phenotypic standard deviation of each trait. The directions of the effects are consistent with the phenotypic correlation between the traits. We are in the process of gathering data regarding sexual maturity and body weight measured later in life cycle of rainbow trout and this study is a first step to further characterise covariation in the genetic architecture of life history and production traits using molecular markers.

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