Influence of a growth hormone transgene on the genetic architecture of growth-related traits in Coho salmon

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Summary

Genetic engineering has been increasingly applied to many commercially important plant and animal species, generating phenotypic changes that are not observed in natural populations and creating genetic interactions that have not experienced natural selection. The degree to and way in which such human-induced genetic variation interacts with the rest of the genome is currently largely unknown. Integrating such information into ecological and risk assessment is crucial to understand the potential effects of genetically modified organisms in natural environments. Here, we performed QTL mapping to investigate the genetic architecture of growth-related traits in non-transgenic (NT) and growth hormone transgenic (T) Coho salmon with large changes in growth and related physiology, with the aim of identifying how an inserted transgene might influence the opportunity for selection. These fish shared the same parental genetic background, thus allowing us to determine whether the same or different loci are involved these traits within the two groups. The use of over 1700 loci, generated through sequencing of restriction site associated DNA markers, revealed that different genomic regions were linked with growth over time between the two groups. Additionally, the effect sizes of specific loci expressed temporally appear to have been influenced by the transgene. Direct comparison of QTL between the T and NT fish during two physiologically matched periods identified little overlap in their location. Taken together, the results showed that the transgene altered the genetic basis of growth-related traits in this species. The study has important implications for effective conservation and management of wild populations experiencing introduction of transgenes. Evolutionary changes and their ecological consequences may occur at different rates and in different directions in NT versus T individuals in response to selection. Assessments of phenotypic change, and hence ecological risk, should be determined periodically to evaluate whether initial estimates made with founder strains remain valid

Keywords: Kodama et al., transgenes, QTL, growth hormone, Coho salmon, risk assessment

Introduction

Recently, variation has been created in many plant and animal species through genetic engineering, generating genetic interactions and phenotypic changes not observed in natural populations. The degree to which such anthropogenically introduced variation interacts with the rest of the genome is often unknown. Understanding such interactions can inform the potential evolutionary effects of genetically modified organisms in natural environments, permitting the integration of such information into ecological and risk assessment frameworks.
crucial for effective conservation and management of wild populations.

Genetic modification of fishes has been underway for more than 30 years, with the primary goals of altering phenotypes for enhancement of production efficiency in aquaculture, applications in basic science, development of strains for the aquarium trade, and control of invasive species (Devlin et al. 2015). Significant potential exists for this technology. However, in some cases such as growth elevation by growth hormone (GH) transgenes, very dramatic phenotypic transformations have arisen. These changes have resulted in public and scientific concern on the potential ecological and evolutionary effects were such animals to enter natural ecosystems. It is critical to know the stability of the phenotype and genotype of the transformed organism in detail for risk assessments to have a high degree of reliability. For example, effects of transgenes on long term adaptation and fitness could affect population size, and behavioural changes may modify the ability of transgenic organisms to utilize or provide resources to other species. Recent data shows that traits of genetically modified fish can be highly dependent on environmental conditions, and that the relative phenotypic relationship of transgenic and non-transgenic fish can vary dramatically among conditions (genotype × environmental responses) (Sundström et al. 2007).

Experiments examining effects of growth-related transgenes in different strains suggest that the role of genome-level influences on phenotype of transgenic organisms may be common (Devlin et al. 2009, Devlin et al. 2013), and it is important to extend this knowledge by elucidating the influence that an inserted transgene may have on individual loci underlying complex phenotypes such as growth, and to determine whether the same or different loci are involved in these traits in transgenic and non-transgenic organisms. This knowledge will contribute to an understanding of how an inserted transgene might influence phenotypic variability and the opportunity for selection should transgenic individuals enter the wild. Here we take advantage of a unique experimental population in Coho salmon to investigate the genetic architecture of growth-related traits in non-transgenic (NT) and growth hormone (GH) transgenic (T) using QTL mapping. Individual fish in the population shared the same parental genetic background and experienced large variation in growth and related physiology (Devlin et al. 2004). Therefore, we were able to examine genomic interactions between transgene and genetic background in NT and T individuals. Specifically, we examined whether QTL affecting variation in growth (body size) of T salmon are shared with, or are distinct from, variation affecting growth in NT salmon.

**Material and methods**

Growth hormone transgenic Coho salmon were obtained from a strain (M77) containing the OnMTGH1 gene construct stably integrated at a single insertion site in a wild-type genetic background (Devlin et al. 2004, Phillips and Devlin 2009). This strain results in a large acceleration of growth under aquarium conditions relative to wild type (Devlin et al. 2015) The strain has been propagated for ten generations via repeated backcrosses of hemizygous transgenic males to wild-type females obtained from the progenitor strain at each generation to maintain the transgene in a wild genetic background. A single-pair backcross family was created by crossing a wild female × hemizygous transgenic male, containing a 1:1 ratio of non-transgenic (NT) and transgenic (T) siblings. At six months, the fish were separated into slow-growing NT fish (confirmed by genotyping) and fast-growing T fish. Each individual fish was tagged, and weighed and measured for length at several periods during their development. The objective was to match NT fish to the T fish at same body size, as a
physiological indicator of developmental stage, rather than at the same time period. Daily growth coefficients (DGC; known to be relatively independent of initial body weight) at each time interval was calculated for all individuals in each family (Dupont-Nivet et al. 2010).

Fish were selected from the upper and lower tails of the length distributions both for NT and T modal groups to enhance the phenotypic divergence among samples analyzed within the resources available. DNA was extracted, restricted with ShfI, and submitted for sequencing (Baird et al. 2008) based on protocols previously described in Kodama et al. (2014). Genotyping was performed following the methods in Kodama et al. (2014).

Quantitative trait locus (QTL) analyses for all traits were performed using single-, two- and multiple-dimensional QTL models in the R-based software package, R/qtl (Broman et al. 2003). The female consensus linkage map for Coho salmon developed by Kodama et al. (2014) was used as a framework for the analyses; since this published map was constructed using the individuals in the current study, polymorphic loci observed in this study were captured in the map. We used the multiple imputation approach to perform quantitative trait loci analyses for all traits (Broman and Sen, 2009), as described in Kodama et al. (2017). The cross was outbred in nature, and so QTL analyses were conducted separately for loci segregating in the male and female parents, so that all marker cross types could be utilized. The chromosomal location of the transgene was identified by linkage mapping with ONEMAP 2.0-3 (Margarido et al. 2007) using loci that were polymorphic in the hemizygous father.

**Results and Discussion**

The aim of the study was to determine whether the insertion of a growth hormone transgene influenced the genetic architecture underlying growth phenotypes in Coho salmon relative to non-transgenic individuals. This aim was achieved by mapping QTL in 243 offspring (121 in the NT group and 122 in the T group) that differed only in the insertion of the transgene, and by comparing loci at shared physiological stages. There were 1784 biallelic loci from the dam and 908 loci from the sire that segregated in the offspring and had known positions on the genome map. The transgene mapped to a metacentric chromosome Co13. There were only two physiologically comparable stages; the first just after smoltification, and the second at maturation.

Broadly, QTL that were polymorphic in the sire were observed in both the transgenic and non-transgenic offspring, but differed in their location (Figure 1). Dam QTL were detected primarily in the transgenic offspring but rarely in the non-transgenic fish. QTL of larger effect sizes were observed in both T and NT offspring, but were more frequent in the former. Four out of seven large effect loci in the transgenes mapped to one region on Co30 and were explained by variation in the sire (Figure 1). Several QTL were shared temporally across traits within, but not between, the T and NT groups. Notably, polymorphic loci in the sire were temporally expressed on linkage groups Co30 and 23 in the T offspring, but on Co15, 20 and 25 in the NT offspring (Figure 1). Polymorphic loci that were attributed to Dam were temporally expressed on Co03 and 04 in the T group only. Comparison between physiologically matched individuals at smoltification showed that almost all QTL that were detected were not shared between T and NT offspring. We conclude that the insertion of the transgene affected both the expression and the effect sizes of QTL involved in growth related traits, thus influencing the phenotypic variance and opportunity for differential selection between T and NT individuals.
Figure 1. Comparison of QTL positions between non-transgenic (NT) and transgenic (T) siblings, produced from a cross between a wild-type Dam and a hemizygous Sire with a growth hormone transgene. Shown are growth-related QTL mapped across different time periods (in blue), using segregating markers from the Sire. Positions of QTL are denoted by Coho salmon linkage groups (Co01-30, Kodama et al. 2014) and nearest marker (letters denote shared positions). Size-matched periods between NT and T groups are periods 1 and 3 for length and 1 and 4 for weight.

Since all fish in this study were derived from the same genetic background, a paucity of common QTL detected between T and NT groups likely arose from interactions between the transgene and other loci in the genome affecting growth; such effects were not confined to the inserted gene itself. Alternatively, if the QTL are shared (and were not detected in one group due to the power of the study), the transgene likely influences the effect sizes of these loci through interactions we were unable to study. These findings support previous observations of large differences in expression levels between NT and T fish of the same strain (Devlin et al., 2013, 2009)(Kim et al. 2015). One of the most interesting results of this study was many larger effect loci were localized on Co30 (the sex chromosome) in T fish, suggesting that the transgene may have influenced the effect size of one or more loci on this chromosome.

The findings reported here provide important insights into understanding how a transgene may influence a species’ response to selection in natural environments. The phenotype of GH transgenic salmon can clearly be influenced by background genetic variation, and as such, selection acting on this variation in nature would be expected to alter the phenotype of T fish in directions that would change their fitness. It seems likely that the T
phenotype could shift over time from when the strain was originally synthesized, influencing the accuracy of risk estimates (Ahrens and Devlin 2011). This study also has important implications for aquaculture if GH transgenic strains were to be used in the future. Because multiple genomic regions other than the transgene have been shown to play an important role in determining growth of T fish, it is important to consider the level of genetic variation in these regions to determine if selection of desired phenotypes could still occur when performing selective breeding.

This study offered an interesting model to examine genomic interactions between transgene and genetic background in NT and T Coho salmon; the results demonstrated that genetic changes (i.e. the growth hormone transgene) with powerful effects on phenotype strongly altered the genetic basis of growth-related traits in this species.

References


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