MAPPING OF QUANTITATIVE TRAIT LOCI IN LOBLOLLY PINE AND DOUGLAS-FIR: A SUMMARY[1]

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ABSTRACT

Classical quantitative genetic approaches have yielded a depth of understanding of the heritability and partitioning of variance of important traits in forest trees. Such knowledge has been applied in the genetic improvement of many species. Genetic improvement based on phenotypic selection in forest trees is rather slow however due to the long generation time in trees and the time to phenotypic evaluation. Genetic marker based approaches to tree improvement would increase the speed and precision of breeding. For this goal to be realized, the genes controlling quantitative traits must be identified. We summarize our progress toward achieving this goal based on a series of quantitative trait locus mapping experiments in two important conifer species. The path toward discovery of economically important alleles is now clear.
Key words: QTLs, genomics, quantitative traits

INTRODUCTION

This paper briefly summarizes our efforts, over a 12-year period, to map quantitative trait loci (QTL) in loblolly pine (*Pinus taeda* L.) and coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii* [Mirb.] Franco), the two most important forest tree species of North America. Our reasons for mapping QTLs were two-fold: (1) to better understand the inheritance and architecture of quantitative traits in conifers and (2) to develop technologies for the improvement of economic traits. The first objective has largely been completed, however this knowledge has not yet been applied in a significant way. We discuss the reasons for this and the solutions we are now pursuing in the final section of the paper.

QTL mapping was made possible in the late 1980s because of developments in DNA marker technology and genome mapping. Genetic maps have been constructed for more than 20 forest tree species and in many cases have been used to map QTLs (http://dendrome.ucdavis.edu/treegenes). In a recent review on QTL mapping in forest trees, SEWELL and NEALE (2000) formed two major conclusions: (1) sufficient verification has not yet been performed in forest trees to assess QTL stability across years (time), environments (space) and family (genotype) and (2) methods other than QTL mapping will be needed to identify genes underlying QTLs and bring marker-assisted breeding to its fullest potential. We have now completed a series of verification experiments in both loblolly pine and Douglas-fir such that we can more confidently assess QTL stability. We have also established a new generation of mapping experiments that we believe will lead to the identification of genes underlying QTLs.

SEWELL and NEALE (2000) described many of the commonly used experimental approaches to QTL mapping in forest trees. Our studies in loblolly pine and Douglas-fir shared a similar approach. Three-generation mapping pedigrees and co-dominant markers (RFLPs) have been used in both cases. Clonal propagation of mapping population progeny was used in Douglas-fir to improve the accuracy of phenotypic evaluations, whereas seedling progeny populations were used in loblolly pine. An all-marker interval mapping approach was developed specifically for QTL mapping in the three-generation pedigrees (KNOTT *et al.* 1997). First-generation detection experiments were performed using existing pedigrees, of relatively small progeny size, occurring in operational progeny test plantations. Subsequent verification experiments were performed using larger populations and test plantations created specifically for QTL mapping. In retrospect, it would have been wiser to perform detection experiments in the larger populations

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and then verify in the smaller operational populations. Nevertheless, it is now possible to view the full series of experiments and attempt to draw conclusions.

WOOD PROPERTY QTL MAPPING IN LOBLOLLY PINE

Through a combination of genetic improvement and silvicultural practices, loblolly pine is now grown on a very short rotation (15–30 years). Rapid growth rates result in much greater yields but also a higher proportion of less desirable juvenile wood. It would be beneficial to have marker-based breeding technologies for wood quality improvement to offset the decline in quality from rapid growth. Furthermore, wood property assessment is often costly and cannot be performed on very young trees. A thorough justification for wood property QTL mapping in loblolly pine can be found in WILLIAMS and NEALE (1992).

QTL discovery

We have mapped QTL for both physical and chemical wood property traits including: (1) earlywood and latewood specific gravity, (2) percent latewood, (3) microfibril angle and (4) lignin, cellulose and hemicellulose content. These traits are all important determinants of lumber strength and/or pulp yield and quality (MEGRAW 1985, ZOBEL & VAN BUIJTENEN 1989, ZOBEL & JETT 1995). A genetic map was constructed for loblolly pine based primarily on RFLP markers (DEVEY *et al.* 1994, SEWELL *et al.* 1999) and was supplemented with ESTP (expressed tag sequence polymorphism) markers in recent years (HARRY *et al.* 1998, TEMESGEN *et al.* 2001, BROWN *et al.* 2001). The most recent version of the genetic map (BROWN *et al.* 2003) is comprised of 12 major linkage groups, presumably representing the 12 chromosomes of loblolly pine, although this has not been established experimentally. The current map is 1305 cM, which represents approximately 75 % genome coverage. There is yet no understanding of the distribution of genes in the pine genome to predict how fully our genetic map includes regions encoding genes for QTLs.

QTL mapping experiments for wood property traits have generally identified 5–10 unique QTLs per trait per family (GROOVER *et al.* 1994, SEWELL *et al.* 2000, 2002, NEALE *et al.* 2002, BROWN *et al.* 2003). Nearly all individual QTLs account for 3–8 % of the total phenotypic variance, with some exceptional QTLs accounting for up to 15 %. The total phenotypic variance accounted for is generally around 25 %, thus assuming heritabilities of around 0.5, we may be accounting for up to 50 % of the genetic variance in any one family. There might be several reasons for not accounting for all the phenotypic variance, such as partial genome coverage and the sizes of mapping populations, however it is most likely that the remainder of the variation is due to a large number of genes with very small effects that are not detectable even in family sizes of several hundred progeny. This description of the architecture of quantitative traits as inferred from QTL mapping is consistent with predictions of polygenic inheritance from quantitative genetic studies.

Both additive and non-additive effects of wood property trait QTLs have been found. This was again expected based on quantitative genetic studies. The refinement gained from the QTL approach is that additive and non-additive effects can be assigned to individual QTLs versus simply estimating the relative proportion of each for the trait as a whole, as is done in the quantitative genetic approach. Knowledge of these differences among QTLs could be important in designing marker-based breeding approaches. It has also been shown that individual trees possess QTLs that both increase and decrease the value of the phenotype. This was also expected because pines are highly heterozygous and not inbred. QTL analyses reveal which QTLs have the positive versus negative effects, whereas quantitative genetic analyses can only estimate the average effect across all QTLs. This too could be important in marker-based breeding.

The distribution of wood property QTLs in the loblolly pine genome appears to be random, although this issue cannot be properly addressed until the genetic map is related to a physical map. A complete genome sequence for loblolly pine may not exist for many years, however, targeted approaches to understanding the "gene space" in pine should be feasible.

QTL verification

As noted earlier, QTL verification has been lacking in forest trees. This is likely due to the time and expense required for such studies and not to a lack of appreciation of its importance. We have performed QTL mapping experiments in multiple years, environments and families such that some conclusions can be drawn about the relative importance of time, space and genotype, respectively in QTL verification (NEALE *et al.* 2002, BROWN *et al.* 2003). Repeatability across years was easily assessed for wood specific gravity and percent latewood because measurements were made on individual annual rings taken from multi-year increment cores (SEWELL *et al.* 2002, BROWN *et al.* 2003). For these traits, about 60 % of

the QTLs were detected in multiple rings. Given that some portion of the 40 % of the QTLs that were detected in only one annual ring might be false positives, these results suggest little temporal variation in QTL expression for these traits. Of course, this generalization will not be true for all traits (see section on Douglas-fir).

Our QTL mapping experiments in loblolly pine did not include clonal progeny populations, thus our power to assess repeatability across environments was limited. Nevertheless, one family was planted at multiple sites in the detection experiment (SEWELL *et al.* 2000, 2002) and on yet a different site in the verification experiment (BROWN *et al.* 2003). Approximately 27 % of the QTLs were detected in both experiments. In spite of the confounding aspects of this comparison, these results suggest a moderate level of repeatability of QTLs for wood property traits across environments. This was expected as G × E is generally not high for wood property traits in loblolly pine. However, QTL × E interactions could be found for a subset of QTLs (SEWELL *et al.* 2002). This again highlights the resolving power of QTL analyses as G × E interactions can only be attributed to traits, and not individual QTLs, using the quantitative genetics approach.

When mapping QTLs in populations created from mating of inbred and highly divergent lines, it is reasonable to assume that most QTLs affecting the quantitative trait will be segregating and will thus be detectable. This assumption cannot be made for most forest tree QTL mapping experiments. Each family will have a subset of QTLs segregating and detectable. This raises the question of QTL repeatability across families (genotypes). We found that approximately 33 % of the QTLs were common to each of two unrelated crosses. This was also expected based on the above assumption. Therefore, QTL discovery must be performed in multiple crosses to enumerate all the QTLs controlling a quantitative trait in forest trees. For these reasons, it is likely that we have yet to discover all the QTLs controlling wood property traits in loblolly pine.

ADAPTIVE TRAIT QTL MAPPING IN DOUGLAS-FIR

Coastal Douglas-fir has a very broad and ecologically diverse range (SILEN 1978). Along the Pacific coast Douglas-fir grows in moist and frost-free environments, however in some parts of its range severe summer drought and early-fall and late-spring frosts are common. Common garden studies have established clear patterns of adaptation across these heterogeneous environments (CAMPBELL & SUGANO 1975, AITKEN & ADAMS 1996, 1997, O'NEILL *et al.* 2001). The delineation of seed zones and development of seed transfer guidelines depend on the understanding of these patterns of adaptation. The specific genes responsible for these adaptations are not known. We have completed a series of QTL mapping experiments for adaptive traits in Douglas-fir with the ultimate goal of identifying such genes. This will enable marker-based approaches for adaptive trait breeding and for guiding conservation of genetic resources.

QTL discovery

Our initial QTL discovery experiment was performed using a three-generation pedigree consisting of clonally replicated progeny. A genetic map was constructed using RFLP and RAPD markers (JERMSTAD *et al.* 1998). The map was comprised of 17 linkage groups ($2x = 2n = 26$) and covered 1062 cM. A framework set of 74 evenly distributed and highly informative RFLP markers was used for QTL mapping. The adaptive traits chosen for initial QTL discovery were (1) vegetative bud flush (JERMSTAD *et al.* 2001a) and (2) spring and fall cold-hardiness (JERMSTAD *et al.* 2001b).

Thirty-three unique QTLs were detected for spring bud flush (JERMSTAD *et al.* 2001a). This is a much greater number of QTLs than were detected for any of the wood property traits in loblolly pine. One obvious difference was that QTLs were not only estimated over multiple years but also over multiple test sites in the Douglas-fir experiment. The number of QTLs detected in any one year or test site is more similar to the loblolly pine results. Furthermore, the power to detect QTLs in the Douglas-fir experiment was probably greater due to the clonal replication of progeny and because the mapping population was constructed specifically to maximize segregation for genes controlling bud flush. The size of effects of bud flush QTLs were small (2.5–8.0 %) and were mostly of additive effect. These results support predictions from quantitative genetic experiments that bud flush is controlled by a large number of genes of additive effect.

Fifteen and eleven unique QTLs were detected for spring and fall cold-hardiness, respectively (JERMSTAD *et al.* 2001b). The sizes of QTL effects were again generally quite small (1.4–9.8 %). These QTLs were estimated at just one test site and in a single year, however they were estimated from three different tissue types (buds, needles and stems).

QTLs were generally common to all three tissue types for spring cold-hardiness, but not for fall cold-

hardiness, suggesting that sychronization of hardening occurs more so in the spring than in the fall. A comparison of bud flush QTLs with cold-hardiness QTLs revealed greater similarity between spring cold-hardiness and bud flush than between fall cold-hardiness and bud flush. This observation is supported by genetic correlations between these traits (AITKEN & ADAMS 1997). The QTL approach, however, reveals which of the individual genes might be responsible for the correlations and thus the molecular basis the of pleiotropic effect.

Growth rhythm traits in Douglas-fir, such as bud flush and bud set, are strongly adapted to seasonal cycles and specific environmental signals such as photoperiodicity, temperature and moisture availability. QTL mapping experiments performed under field conditions do not reveal interactions between QTLs and specific environmental signals. To address these questions, we performed another QTL discovery experiment using experimental treatment of environmental signals (JERMSTAD *et al.* 2003). A second clonal progeny mapping population (N = 429) was developed from the same three-generation pedigree as was used in the earlier QTL discovery experiments. Two QTL mapping experiments were performed. A growth initiation experiment was designed to identify QTLs for bud flush interacting with winter chill and spring flushing temperatures. A growth cessation experiment was designed to identify QTLs for a suite of growth rhythm traits, including the date of bud set, interacting with daylength and moisture stress. The goal of these experiments was to identify QTLs that interact with these specific environmental signals and that would guide us in the identification of candidate genes underlying the QTLs.

The number of QTLs detected per trait, the sizes of their individual effects and proportion of additive versus non-additive effects were consistent with our earlier QTL discovery experiments in Douglas-fir. In this experiment, however, a subset of QTLs was shown to have interactions with one and sometimes both of the experimentally applied treatments. For example, a QTL for bud flush on linkage group 2 interacted with winter chilling. This suggests that the gene underlying this QTL may be involved in winter bud dormancy release. In the growth cessation experiment, there was a QTL for bud set on linkage group 11 that interacted with daylength. The gene underlying this QTL is likely to be involved in detecting photoperiodic stimuli such as the phytochrome genes. Although much more work will need to be done to identify genes controlling adaptive traits in Douglas-fir, these QTL mapping experiments have taken a big step toward dissecting these complex traits down to their individual parts.

QTL verification

Our QTL mapping experiments in Douglas-fir have employed clonal mapping populations, thus enabling verification of QTLs across years and environments. However, all experiments involved just one three-generation pedigree, so verification across families has not been possible. QTLs for spring bud flush were measured in four consecutive years (JERMSTAD *et al.* 2001a) and a high level of repeatability was observed. No other traits have been measured in multiple years in the Douglas-fir experiments. We expect that temporal repeatability will be high for some traits such as bud phenology and wood properties, but may not be for traits such as height or diameter growth (KAYA *et al.* 1999). QTL repeatability was nearly absent for bud flush between two different test sites (JERMSTAD *et al.* 2001a). However, we speculated that this was likely due to the much lower power of detection at one of the sites. QTL repeatability for bud flush across sites was tested again in the second experiment (JERMSTAD *et al.* 2003), and this time repeatability was very high. We also expect that spatial repeatability will vary among traits and environments.

SEARCHING FOR GENES UNDERLYING QTLs

QTL mapping provides reasonable estimates of the number, genomic location and size of effect of genes controlling complex traits. Furthermore, the QTL approach searches the entire genome (genomic scan) for the presence of QTLs, assuming that the map provides full genome coverage. However, QTL mapping does not identify the specific gene underlying the QTL. In fact, the QTL positions are not highly resolved. The interval within which a QTL is normally mapped in a forest tree might include hundreds of genes. This precludes using genetic markers flanking QTLs for family selection in trees because linkage equilibrium conditions between marker alleles and QTL alleles should be assumed (STRAUSS *et al.* 1992). The size of intervals could be reduced by increasing the number of recombinants in mapping populations, but this requires thousands, if not tens of thousands, of progeny. Although possible, this is not a tractable approach. An alternative approach, called association mapping, takes advantage of historical recombination that has occurred in a population. This approach is used widely in human genetics to dissect complex diseases (RISCH 2000) and is now being applied to agricultural plants and animals (RAFALSKI

2002), including forest trees (NEALE & SAVOLAINEN 2003).

The association approach to dissecting and mapping complex traits is very similar to the QTL approach, except for the genetic structure of the mapping population. Members of an association mapping population are generally unrelated and are sampled from large random mating populations. Many forest tree species are ideal for association mapping because it is easy to sample large numbers of trees from random mating populations (NEALE & SAVOLAINEN 2003). Greater precision of evaluating the phenotype is possible if sampled trees are clonally replicated or progeny tested.

We are using candidate gene based association mapping to identify the genes underlying QTLs for wood property traits in loblolly pine and adaptive traits in Douglas-fir. A candidate gene approach is an alternative to the full genome scan that is now being used in humans and a few plant and livestock species. Genome scans require very dense genetic marker maps based on single nucleotide polymorphisms (SNPs). Dense SNP maps are very expensive to develop and are not feasible for forest trees at present. Thus, associations are currently limited to candidate genes that are expected to have some effect on the phenotype. Candidate genes can be identified based on (1) their function in model systems, (2) their map position relative to QTLs or (3) their patterns of gene expression. We are using these criteria to develop extensive candidate gene lists for wood property and adaptive traits.

Once candidate genes are identified, DNA sequences are determined from a small sample of individuals to discover SNPs in the population. Once SNPs are found, all members of the association population are genotyped for the desired SNPs. Large (500–1000) clonally replicated or progeny tested populations have been developed and outplanted to replicated genetic tests. Wood property and adaptive trait measurements will be made in these tests. Finally, statistical tests of association between SNP genotype and quantitative trait value can be made. Positive associations indicate that the candidate gene has an effect on the quantitative trait.

The association mapping approach has great potential to identify the genes underlying complex traits in forest trees. Gene sequencing projects in loblolly pine (KIRST *et al.* 2003) and other forest trees have produced a catalog of DNA sequences of many of the genes found in trees. From this resource, candidate genes for almost any complex trait can be found. The selection of the most important candidates, among the 30,000+ genes that are expected in the genome, remains an imperfect task. Nevertheless, DNA sequencing and high throughput SNP genotyping technologies are developing rapidly such that it should be possible to screen hundreds if not thousands of candidate genes simultaneously for associations with any trait. The physical distance within a gene between the SNP and the causative mutation will be very small, such that recombination may never have occurred between the two polymorphisms in the evolutionary history of the tree species. Genetic markers of this type could be used in any population to select for economically important alleles and germplasm conservation. At such a time, the final limitation to marker-based breeding might be overcome.

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