

QTL MAPPING IN INTERSPECIFIC HYBRIDS OF FOREST TREES

Harvey D. Bradshaw, Jr.¹ & Dario Grattapaglia²

¹Center for Urban Horticulture, Box 354115, University of Washington, Seattle WA 98195–4115, U.S.A

²CENARGEN–EMBRAPA, SAIN Parque Rural, C.P. 02372, Brasilia D. F. 70879–970, Brazil

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ABSTRACT

Interspecific hybridization leads to heterosis for growth in a significant number of forest tree genera. When it is possible to exploit this heterosis by vegetative propagation, as is currently the case in the hardwood genera *Eucalyptus* and *Populus*, interspecific hybridization is often the preferred route to genetic gain. The genetic basis of variation among hybrid offspring and the genetic mechanisms underlying heterosis observed in interspecific hybrids in forest trees are poorly understood, however. A deeper understanding of the genetic control of growth in interspecific hybrids will be necessary to design and implement long-term breeding strategies. We have produced linkage maps of F₁ and F₂ interspecific hybrids in *Eucalyptus* and *Populus*, respectively, in order to address these unresolved issues. We have identified quantitative trait loci (QTLs) with major effects on stem growth, form, leaf phenology, wood quality, disease resistance, and organogenesis *in vitro*. Patterns of QTL inheritance for gross phenotypic traits, *e.g.*, stem volume growth and circumference, have been compared with QTLs identified for physiological and anatomical correlates of these traits, *e.g.*, sylleptic branch leaf area and wood specific gravity. Co-localization of QTLs suggests novel approaches to indirect selection in clonally propagated interspecific hybrids. Significant questions of QTL constancy across families, tree age, and environment remain to be studied.

Key words: *Eucalyptus grandis*, *Eucalyptus urophylla*, *Populus trichocarpa*, *Populus deltoides*, heterosis, genome

INTRODUCTION

Heterosis for vegetative growth is characteristic of interspecific hybrids and/or wide crosses in many plant genera; forest trees are no exception (WRIGHT 1976; ZOBEL & TALBERT 1984). Exploiting the phenomenon of heterosis requires either a simple method for generating large numbers of hybrid seedlings, analogous to male sterility and restoration of fertility in hybrid maize, or the ability to vegetatively (*i.e.*, clonally) propagate desirable hybrid genotypes. In current forestry practice, only clonal propagation has been widely used to capture heterotic combinations for commercial planting, largely limiting the application of interspecific hybridization to those taxa with well-developed vegetative propagation systems, such as *Eucalyptus*, *Populus*, and *Salix*.

Heterosis cannot result from purely additive modes of gene action, and so it is not surprising that genetic improvement strategies designed to capture additive genetic variance, as implemented in most conifer breeding, may be inappropriate for interspecific hybrids. In order to design and implement genetic improvement strategies tailored to the idiosyncrasies of interspecific hybrids in forest trees, basic information is needed on the genetics of variation in commercially-

important traits. In particular, we have focused on two questions:

1. Which genetic loci are responsible for quantitative variation in commercial traits among F₁ hybrid offspring? The variance among F₁ hybrid clones is the typical substrate for selection in most hybridization programs, making this question of obvious practical significance. Genome mapping in *Eucalyptus* hybrids has been used to address this topic in forest trees.

2. Which genetic loci are responsible for F₁ heterosis? This question is best studied by identifying loci contributing to genetic variance in the F₂ or backcross generations, as has been done in *Populus*. The contribution (positive or negative) and mode of action of each parental QTL allele to the phenotype of the hybrid may be determined, and used to evaluate the merits of various long-term breeding plans.

The purpose of this review is to summarize the current state of our understanding of quantitative trait inheritance, as derived from genome mapping studies in interspecific hybrids of forest trees, and to indicate research directions for the immediate future. Specific examples will be drawn from the authors' experiments in two genera of fast-growing hardwoods, *Eucalyptus* and *Populus*; however, many of the general conclusions

should apply equally well to interspecific hybrids in other angiosperms, such as *Platanus* and *Salix*, as well as to gymnosperm genera, such as *Larix*, *Picea*, and *Pinus*. Whether patterns of quantitative trait inheritance deduced from studies in interspecific hybrids are relevant to those found within a single species remains an open question.

RESULTS OF QTL MAPPING IN INTERSPECIFIC HYBRIDS OF *EUCALYPTUS*

The principal objectives of the *Eucalyptus* research reviewed here were to test the hypothesis of the existence of discrete QTLs with major effects controlling the variation in quantitatively inherited, economically-important traits and to test the full- and half-sib approaches for QTL mapping in forest trees, using dominant RAPD markers (WILLIAMS *et al.* 1990) segregating in a pseudo-testcross configuration in a two-generation pedigree. Detailed descriptions of this research have been published (GRATTAPAGLIA 1994, GRATTAPAGLIA & SEDEROFF 1994; GRATTAPAGLIA *et al.* 1995).

A full-sib family of 122 individuals was generated from a single controlled cross between two highly heterozygous elite trees. A plus tree of *Eucalyptus grandis*, used as the female parent, was mated with a plus tree of *E. urophylla*. Many of the commercial clones used in the tropics are derived from this same *E. grandis* female. The F_1 hybrid population was immortalized by establishing clonal cultures of the individuals by vegetative propagation. A second QTL mapping population of 300 F_1 hybrids, already at rotation age (6.5 years), consisted of an open-pollinated maternal half-sib family of the same plus tree of *Eucalyptus grandis*. The pollen parents of the half-sib family are 24 male *E. urophylla* in the seed orchard with the *E. grandis* female.

In the full-sib family, a total of ten putative QTLs were mapped for micropropagation response (fresh weight of micropropagated shoots; SHOOT), six for sprouting ability (number of commercial cuttings obtained after coppicing; CUT) and four for rooting ability (percentage of cuttings forming roots; ROOT). For SHOOT, individual QTLs explained 6.8 – 12.3% of the phenotypic variance, for CUT estimates were between 7.3 – 10.8%, and for ROOT estimates were from 7.3 – 21%. Simultaneous multilocus estimates of the total proportion of phenotypic variation explained by the joint action of the putative QTLs mapped for each parental tree were 52.5 % for SHOOT, 28.2 % for CUT, and 32.6 % for ROOT.

Differences in least square mean trait value between the two alternative QTL genotypes (D_1) ranged from 0.46 to 2.1 phenotypic standard deviations (σ). For all the putative QTLs detected, variances of the alternative

QTL genotype classes were generally equal and close in value across QTLs within traits. Estimates of D_1 were 0.5 – 0.7 σ for the majority (65%) of the QTLs detected. A potentially more interesting estimate from the breeding standpoint is the difference in mean trait value between the family mean and the favorable QTL genotype (D_2). These differences ranged from 0.25 – 1.07 σ and typical values were between 0.2 – 0.4 σ . The QTL detected with the highest LOD score (5.8) was for ROOT in *E. urophylla*. The effect of the substitution of the linked RAPD marker resulted in the doubling of the rooting percentage (23.4 – 48.3%) or an increase in 12% rooting above the family mean ($D_2 = 0.58\sigma$). QTLs of large effects for SHOOT were detected for both parents, and in both cases the difference between the favorable allele and the family mean were estimated to be above 1.0 σ .

In both species, there were two cases where the LOD 1.0 support interval of QTLs detected for SHOOT and CUT overlapped. Both traits involve multiple shoot formation from dormant buds, differing in the fact that in micropropagation response, shoot formation is stimulated *in vitro* with the action of cytokinin while in number of cuttings it relies exclusively on the intrinsic physiological ability to break dormancy of resting juvenile buds. It seems reasonable to suggest that these two traits, although not significantly correlated in this experiment, should share some common QTLs. Although pleiotropic gene action might be a possible explanation for the coincidence of these QTLs, at this point we cannot distinguish between pleiotropy and tight linkage of different QTLs. However, the sharing of some QTL regions could be interpreted as an indirect biological validation for these QTLs.

In the half-sib family, a total of 12 QTLs were detected controlling an estimated 11% of the phenotypic variation for circumference at breast height (CBH; 3 QTLs), 25% for wood specific gravity (WSG; 5 QTLs) and 12% for percent dry weight of bark (BARK; 3 QTLs). For pulp yield (PULP) only one QTL was detected. QTL detection power was considerably lower for PULP as the sample size used was only 164 individuals and the trait heritability is typically low (0.23; DEMUNER & BERTOLUCCI 1993). Estimates of the difference in least square mean trait value between the two alternative QTL genotypes (D_1) ranged from 0.26 to 0.51 phenotypic standard deviations (σ) and most of the values were around 0.4 σ . Differences in mean trait value between the family mean and the favorable QTL genotype (D_2) ranged from 0.10 to 0.27 σ and typical values were around 0.2 σ . The great majority of the QTLs mapped to four linkage groups. Overlapping QTLs for CBH, WSG, and BARK were observed on a single linkage group where either one or more genetic loci with pleiotropic effect or a cluster of linked genes

control all three traits. Overlapping of QTL positions was also observed for CBH and WSG on two additional linkage groups and for WSG and BARK on yet another. Simultaneous QTL mapping by multivariate regression detected a significant case of digenic epistasis ($P < 0.01$) between unlinked QTLs, that increased the total proportion of the phenotypic variance explained in CBH from 11 to 15% (GRATTAPAGLIA *et al.* 1995).

RESULTS OF QTL MAPPING IN INTERSPECIFIC HYBRIDS OF *POPULUS*

QTL mapping experiments in *Populus* share some objectives with parallel work in *Eucalyptus*, in particular the demonstration that QTLs responsible for genetic variation in commercially-important traits can be mapped in a forest tree. In addition, by using a three-generation pedigree derived from interspecific hybridization across taxonomic sections in *Populus*, it was hoped that insights into the genetic basis of hybrid vigor itself might be found (BRADSHAW & STETTLER 1995).

The *Populus* QTL mapping pedigree was constructed by hybridization between *P. trichocarpa* and *P. deltoides*. The predictable heterosis in this hybrid combination is the basis of applied genetic improvement programs for *Populus* in much of western North America. Mating of two F_1 siblings was performed to produce the F_2 . Fifty-four F_2 clones, along with the F_1 and parental trees, were established in a pilot-scale replicated clonal trial (6 ramets per clone) in 1990. Phenotypic data from the clonal trial can be used to calculate broad-sense heritabilities for all traits, allowing us to estimate the proportion of genetic variance accounted for by each mapped QTL.

More than 100 phenotypic traits have been measured in this clonal trial, including height, diameter, and biomass growth; harvest index (based on a thinning after three years, or half of rotation age); stem taper; branch type, number, length, and angle; and leaf phenology, color, size, shape, and distribution among branch types. The current linkage map for this F_2 family consists of 343 RFLP, STS, and RAPD markers and covers approximately half the genome (BRADSHAW *et al.* 1994). Unlike the *Eucalyptus* maps, which depend upon heterozygosity within an individual tree segregating in an F_1 , the *Populus* map is constructed from marker segregation in the F_2 by treating the parental species as homozygous inbred lines and the F_1 sib-mating as a self-fertilization. Only two alleles, derived either from *P. trichocarpa* (T) or *P. deltoides* (D), were recognized at any locus, which has the potential to downwardly bias estimates of QTL magnitude (BRADSHAW & STETTLER 1995). The tacit assumption is that most of the genetic variance for the traits of interest is partitioned between the parental species rather than among

individuals within the species. This assumption is justified by the observation that F_1 interspecific hybrids invariably outgrow the better parent; hence, the major genetic determinants of F_1 heterosis appear to be fixed in the parental species. The theoretical disadvantage of treating the parental clones as inbred lines is clear: *Populus* clones, like most trees, are not homozygous but highly heterozygous. The advantage of treating the two parental species as inbred lines is that any marker which is polymorphic between the two parental species is informative for QTL mapping under the "simplified" model, and such "fixed" polymorphisms are more abundant than the multi-allelic markers necessary for a more thorough analysis of QTL inheritance.

The most comprehensive analysis of growth, branch, and leaf traits was carried out two years after plantation establishment, one-third of a typical rotation for pulpwood. Some examples of mapped QTLs follow. For second-year height, a single QTL was found accounting for 26% of the phenotypic variance and 32% of the genetic variance, with the T allele having a positive effect dominant to the D allele. The difference (D_1) between the phenotypic means of the alternate homozygotes is 4σ . Second-year basal area is partly controlled by a single QTL explaining 24% of the phenotypic variance, 38% of the genetic variance, having the D allele dominant with a positive effect, and a D_1 value of 0.9σ . A two-QTL model for stem volume (height * basal area) explains 29% of the phenotypic variance and 45% of the genetic variance in the F_2 . Height:diameter ratio (HDR; a surrogate for stem taper) was influenced by a single QTL responsible for 39% of the genetic variance, with the T allele increasing HDR and dominant to the D allele. The date of spring leaf flush, associated with tolerance to late frosts, had the highest broad-sense heritability of any trait measured ($H^2 = 98.0\%$). Five QTLs with major effects on the timing of spring bud break were identified. The QTLs were distributed among five different linkage groups each accounting for 28.7 - 51.5% of the phenotypic variance. Three of the QTLs showed a dominant mode of action, with early flush dominant to late flush regardless of the origin of the allele (T or D). The two remaining QTLs apparently are additive in effect. At four of the five QTLs the T allele conditioned early leaf flush, as expected from the mild climate from which the *P. trichocarpa* female parent originated. When all five QTLs are combined in a single model, 85% of the genetic variance is explained. Genotypic data from the marker nearest each QTL were used to test for epistasis, but no significant interaction term ($P < 0.05$) was found.

The stem basal area QTL is flanked by QTLs for sylleptic branch total leaf area (sylleptic branches arise from buds that have not overwintered), sylleptic branch number, and sylleptic branch leaf number. At all of

these QTLs the D allele has a dominant positive effect on the phenotype. The coincidence of map position and mode of action suggests that that pleiotropy of a single QTL is probably responsible for the QTL 'clustering', and is more likely than linkage to explain the phenotypic correlations between radial growth and sylleptic branch leaf area.

WHAT HAVE WE LEARNED FROM EARLY QTL MAPPING WORK IN INTERSPECIFIC HYBRIDS?

Interspecific hybrids of forest trees are suitable for studying quantitative trait inheritance

As with herbaceous plants (*e.g.*, PATERSON *et al.* 1988, KEIM *et al.* 1990), interspecific hybrids of forest trees have proven to be especially amenable to map-based QTL analysis. There are several reasons for this. First, *Eucalyptus* and *Populus* hybrids are fast-growing trees, typically harvested after only 4–10 years of growth. Commercially-important phenotypes are revealed soon after planting, and the problem of poor juvenile-mature phenotypic correlations is minimized. Second, these hybrids are usually grown in a plantation culture system with a relatively homogeneous environment, resembling agriculture more than traditional extensive forestry. This reduces the non-genetic variance components of complex traits like stem volume growth, and facilitates detection of the genetic basis of phenotypic variance. Third, clonal propagation allows precise estimation of the broad-sense heritability and an accurate assessment of the magnitude of effect of QTLs on the genetic variance.

Our experiments have shown that QTLs for economic traits may be mapped in all types of pedigrees used in operational tree improvement involving interspecific hybrids: full-sib F_1 s, half-sib F_1 s, and advanced-generation (F_2) progenies.

QTLs causing variation in the F_1 generation can be mapped

In interspecific F_1 families, such as the two-generation *Eucalyptus* pedigrees described above, the linkage disequilibrium between molecular marker and QTL alleles required for mapping is produced by heterozygosity in each parental tree. Parental heterozygosity is also responsible for the phenotypic variation among F_1 offspring that is tapped for many genetic improvement strategies. Hence, favorable QTL alleles mapped in the F_1 generations are of immediate interest as targets of

clonal selection in those families where marker/QTL associations have been established. It is generally possible to produce very large full- or half-sibships, so a high selection intensity could be applied at the seedling stage using the appropriate markers to identify desirable genotypic configurations among the progeny.

The genetic basis of heterosis in interspecific hybrids of *Populus* appears to be dominance of complementary QTL alleles from each parental species

Interspecific F_1 hybrids of *P. trichocarpa* and *P. deltoides* show predictable heterosis for growth. The QTLs responsible for this heterosis segregate in the F_2 generation, as they do in advanced generations of maize (STUBER *et al.* 1992). Analysis of recombination between the genomes of *P. trichocarpa* and *P. deltoides* indicates that the fundamental basis of F_1 heterosis is the complimentary action of dominant alleles for increased height from *P. trichocarpa* and for increased radial growth from *P. deltoides*. This suggests new approaches to poplar breeding, including the use of advanced generation (F_2 , F_3 , etc.) offspring selected based on a "marker ideotype", and perhaps the introgression of desirable QTL alleles from one species into the other by backcrossing.

Coincidence of QTL position, magnitude of effect, mode of action, and direction of allelic effect suggest that pleiotropy can explain correlations among anatomical, physiological, and gross morphological traits

Tree physiologists and anatomists have spent many years searching for predictors of tree growth and wood quality that could be applied to early selection of desirable genotypes. With QTL mapping, we are able to provide a more detailed genetic explanation for the physiology of growth and the anatomy of wood. Examples of co-localization of QTLs for tree growth and correlated traits (*e.g.*, wood specific gravity and sylleptic branch leaf area) are found in *Eucalyptus* and *Populus*. The ability to map QTLs for morphophysiological traits also suggests that molecular breeders will be able to provide tree physiologists with subsets of clones differing primarily at a single QTL, with other loci held constant. This highly refined material is the forest tree equivalent of near-isogenic lines available in inbreeding crops, and will tremendously increase the power of physiological studies.

WHAT ARE THE HIGHEST PRIORITY RESEARCH ISSUES IN INTERSPECIFIC HYBRID SYSTEMS?

Stability of QTLs across species

In many genera where interspecific hybridization is an important tool for tree improvement, more than two species are available as parents of the F_1 . In breeding systems that depend only upon clonal selection among F_1 hybrids, it is not possible to combine the genomes of more than two species simultaneously. It is not known whether QTL alleles important in one interspecific combination are similarly important in other hybrid combinations, or in crosses made within a single species, but such experiments are in progress.

Stability of QTLs across families

We have relatively little information on the conservation of QTL position and magnitude of effect across families. If QTL patterns are vastly different among families, the implication is that complete linkage maps would need to be made for every individual tree in the breeding population in order to apply marker-assisted selection (MAS). The concept of a single unified map for a species and unified QTL information that could be used across individuals, *i.e.*, at the population level, is probably unrealistic as a first approach to QTL mapping and MAS in genetically heterogeneous forest trees. By working with individual maps and individual QTLs, stability of QTLs at the population level is not an issue. However, the expression of a particular QTL allele mapped in one individual tree may vary depending on the existing genetic background. Possible epistatic interactions may lead to a situation where a particular QTL allele contributed by a certain individual A is expressed in its progeny of the cross with an individual B but not when A is crossed to another individual C. This is probably the most important issue that needs to be investigated experimentally if one wishes to consider MAS in operational tree improvement. The finding that QTLs can be mapped in the maternal parent of a large half-sib F_1 family in *Eucalyptus* suggests that at least some QTLs will be stable across a variety genetic backgrounds.

Stability of QTLs across time

Stand development in forest trees takes several to many years, and undoubtedly there are different suites of QTLs active at various life history stages (*e.g.*, establishment, juvenile growth, crown closure and competition, flowering). This is known to be true in *Populus* (BRADSHAW & STETTNER 1995). Quantitative genetic

studies suggest that patterns of genetic variance change over time (BALOCCHI *et al.* 1993). It should prove interesting to see how these changes in variance components occur at the sub-chromosomal level. Further, by mapping QTLs for both incremental and cumulative traits, such as stem growth, it should be possible to more accurately model tree responses to stand development. In clonally-propagated trees such topics as competition can be investigated by replicating test designs at different tree spacing, for example.

Stability of QTLs across environments

Provenance trials in forest trees indicate that there will be substantial environmental variance and genotype \times environment (G \times E) interactions in the expression of most commercial traits. Genome mapping work in crop plants (PATERSON *et al.* 1991, STUBER *et al.* 1992) and loblolly pine (GROOVER *et al.* 1994) supports the notion that some QTLs are important across all environments and others vary in their magnitude of effect in different environments. We have installed experiments in *Eucalyptus* and *Populus* to examine QTL effects in environments contrasting in soil fertility and water availability.

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