ANALYSIS OF GROWTH, FORM AND BRANCHING TRAITS IN AN F2 POPULATION OF THE *Pinus elliottii* x *Pinus caribaea* INTERSPECIFIC HYBRID USING RAPD MARKERS

Glenn Dale [1,2,3] and Bob Teasdalela

Abstract.--The developmental biology of the haploid conifer megagametophyte has been exploited using dominant RAPD markers to construct a reference genetic map for an individual *Pinus elliottii x Pinus caribaea* Fl hybrid tree forming part of a three generation pedigree. This map incorporates 186 markers across 17 linkage groups. At 1595cM, the map is estimated to cover 80% of the genome at an average marker density of 8.6cM. Using this reference genetic map with phase known, genotype information obtained using dominant RAPD markers in the F2 population was successfully used to determine full genotype classification. This genotype information was used to map QTL for five growth and form traits and seven branching traits in six year old trees. A total of 57 putative QTL were identified for all 12 traits examined. Considerable overlap existed between QTL identified for under and overbark diameter and bark thickness, and between QTL for branch angle, regularity of branch spacing, ramicorn number, and occurrence of double leaders, suggesting common genetic control of physiologically related traits. Detailed analysis of QTL for bark thickness indicated cryptic genetic variation not evident from the phenotype of either parent, as well as additive, dominant, overdominant and underdominant modes of gene action. Loci whose effects are stable across environments and specific to particular environments were also indicated. An approach was developed to accumulate confidence in QTL results obtained from small populations: potentially a common limitation to QTL mapping in forest trees. The implications of these findings for both conventional tree breeding and marker assisted breeding are discussed.

Keywords: Genetic mapping, QTL mapping, RAPD, genotyping, F2 intercross, interspecific hybrid, tree breeding, *Pinus elliottii* x *Pinus caribaea.*

INTRODUCTION

The Fl hybrid between *Pinus elliottii* and *Pinus caribaea* is the most economically important forest plantation species in sub-tropical Queensland, Australia. The performance of this hybrid is at least equal, and often superior to the pure species of both its parents in all commercially important traits. Breeding and improvement programs over the past thirty years have achieved significant gains in yield and stem quality of both the parental species, and identified parents with specific hybridizing ability (Nikles and Newton 1991). Gains achieved through breeding have been matched by the development of an operational vegetative propagation system, and practices to maintain juvenility. These combined developments are poised to be capitalized on by a shift from family to clonal forestry (Haines and Walker 1993a).

---

[1] ForBio Research Pty. Ltd., C/- **BSES,** Indooroopilly, Queensland 4068, Australia
[2] Department of Botany, University of Queensland, St. Lucia, Queensland 4072, Australia
[3] Present address: Forest Biotechnology Group, North Carolina State University, Raleigh, NC 27695, USA

In the context of this advanced breeding and propagation program, future gains will be facilitated by a precise understanding of the specific effects and interactions of alleles from each parental species at quantitative trait loci (QTL) influencing commercially important traits. Such knowledge may potentially influence the efficiency and rate of improvement in each parental species, selection of parents from each species for hybridization, within family selection of Fl or F2 hybrid individuals for clonal propagation, and the capacity for prospective breeding of superior hybrids from advanced hybrid individuals.

In an appropriate pedigree, genetic mapping provides a systematic framework for the investigation and quantitation of allelic effects and interactions. In an F2 self or intercross population, the effect of substituting both one and two alleles at a given locus may be followed with co-dominant molecular markers, and hence both additive gene effects and dominance may be quantified. For this reason, Paterson et al. (1991b) refer to an F2 self, segregating in the classical 1:2:1 Mendelian ratio for the three possible genotypes, *as* the 'ideal' population for studying gene dosage effects (or gene action) in diploids. This contrasts with testcross or half-sib populations which only permit characterization of the effect of a single gene substitution at any given locus (Paterson et al. 1991b)

Although the 'ideal' QTL mapping population for studying gene dosage effects and intra-allelic interactions is rare in most commercial tree breeding programs, such a population was available within the Queensland Forest Service tree breeding program. This population thus provided a unique opportunity for QTL investigation in forest trees. The size of this F2 population was restricted to just 54 individuals, far below the few hundred to few thousand estimated to be required to detect QTL effects of 1% to 5% of the phenotypic variance (Weller 1992). Yet this unique population represented a valuable model to gain a preliminary indication of the architecture of the most significant loci influencing a range of quantitative traits in forest trees, the mode of action underlying these loci, an appreciation of the value of an F2 population for QTL mapping and guidance for the experimental design of future studies. Finally, it provided the opportunity to develop an approach to extend the utility of dominant RAPD markers to provide co-dominant genotype information (Dale and Teasdale 1995).

MATERIALS AND METHODS

Experimental Pedigree

A unique three generation pedigree comprising selfed F2 progeny of an Fl individual from an interspecies cross, was identified within the Queensland Forest Service hybrid breeding and evaluation program. *Pinus elliottii* E1-023, planted around 1934, was used as the maternal parent, and *P. caribaea* CH6-029, planted around the early 1950s, was used *as* the pollen or paternal parent. Both parents were selected for intercrossing on the basis of their superior growth and form characteristics relative to other individuals within each species. The Fl hybrid family produced from this cross was planted at Beerwah in 1962. A ramet of one of the plus trees selected from this Fl family, EH4, was selfed to provide F2 progeny, planted at Beerwah (24) and Tuan (32) in May/June of 1987. Of the seedlings planted, 23 remain surviving at Beerwah, and all 32 have survived at Tuan.

Site Description

Temperature and rainfall patterns were similar between sites. Soil type at Beerwah is a deep Red earth, rated as plantability category 'A'. Soil type at Tuan is a Lateritic podzolic, rated as plantability category 'B', inferior to the soil type at Beerwah. Prior to establishment of the experiment, the Tuan site was an improved pasture. Beerwah is a second rotation site, having been under *P. elliottii* and *Pinus taeda* since 1932.

Assessment of Traits

All F2 trees were measured in June 1993 at six years of age for the following quantitative and quasi-quantitative traits: i) over and underbark diameter at breast height, ii) height, iii) average bark thickness, iv) stem straightness, v) number of ramicorns, vi) number of double leaders, vii) average branch angle and viii) regularity of whorl spacing (co-efficient of variation for whorl spacing expressed as a percentage). Branching traits were measured on all branches between breast height (1.3m) and 3/4 of total tree height.

Genetic Mapping of the Fl Hybrid, EH4

A total of 520 RAPD primers (Operon™ kits A to Z, 20 primers per kit) were screened using the Fl hybrid, EH4, and its two parents, E1-023 and CH6-029. RAPD reactions were performed, and marker segregation data collected, on a set of 92 megagametophytes, commencing with the primers generating the highest number and quality of putative polymorphic markers. Linkage analyses for the construction of a genetic map from marker segregation data were performed using Macintosh MapMaker V1.0 (Proctor et al. 1990).

Genotvping of the F2 Population

The dense genetic map constructed for EH4 was used as a reference to select markers for the purpose of determining their pattern of segregation in the F2 progeny of this individual. Where possible, pairs of markers closely linked in repulsion were selected with a spacing of around 20cM between marker pairs. Alternatively, alternating maternal and paternal markers were selected at around 10cM intervals along each linkage block. When feasible, markers were selected to minimize the number of primers and hence RAPD reactions required.

Inference of Fully Classified Genotypes

F2 genotype data was arranged in a spreadsheet with individuals in columns and markers in rows. Markers were arranged in the same order as they occurred in each linkage group. Three contiguous columns were generated for each individual. Maternal genotypes were aligned in the first, and paternal in the second. By reference to a diagram of each linkage group, genotype was inferred for unknown regions of each linkage group within each individual, by assuming a chiasma to occur midway between known parental genotypes of opposite phase. The fully classified inferred genotype of each linkage block within each individual was entered in the third column. This portion of the spreadsheet was later extracted to compile a database suitable for QTL analysis with MapMaker

Proportion of Parental Genome Inherited

Based on the inferred fully classified genotype data, HyperGene™ software (Young and Tanksley 1989, 1991) was used to calculate the proportion of parental genome comprising each F2 individual, the overall average of each parental genome inherited in the F2 population, and the proportion of each F2 individual heterozygous and homozygous for the alternative parental alleles.

Quantitative Trait Analysis

Co-segregation analysis of genotype data with phenotype data to identify and characterize QTL for each trait assessed, was carried out by interval mapping (Lander and Botstein 1989). This procedure was performed using MapMaker/QTL V1.1 (Lincoln et al. 1992). A LOD threshold of 2.6, corresponding to a nominal significance level of about $P = 0.001$ per test or $P = 0.05$ for the entire genome, was used to declare the presence of significant QTL.

RESULTS

Site Effects on Trait Characteristics

Table 1 summarizes for both the Beerwah and Tuan sub-populations, the trait means and co-efficient of variation for all traits examined.

Table 1. Mean, co-efficient of variation and site effects for growth, form and branching traits in selfed F2 progeny of the *P. elliottii x P. caribaea* hybrid.

Site	Tuan		Beerwah		Difference Between Sites
	Mean	Co-efficient of Variation	Mean	Co-efficient of Variation	
Average Bark Thickness (mm)	18.3	17.4	19.0	15.8	NS
Over Bark Diameter (cm)	14.2	12.9	16.4	10.9	*SIG
Under Bark Diameter (cm)	10.6	10.8	12.6	11.8	*SIG
Height (m)	8.2	9.9	10.6	9.7	*SIG
Straightness	2.0	50.0	3.1	27.5	*SIG
Ramicorns per Tree	0.4	186.2	0.3	207.3	NS
Double Leaders per Tree	0.1	387.1	0.0		NS
Branch Angle (° from vertical)	69.0	10.2	71.9	11.7	NS
Regularity of Whorl Spacing	0.6	30.9	0.5	42.8	NS

Genetic Mapping of the Fl Hybrid, EH4

Linkage analysis of 232 Mendelian markers produced a haploid linkage map for the Fl hybrid of *P. elliottii x P. caribaea* incorporating 186 markers across 17 linkage groups varying in size from 7 to 175cM, and comprising a total haploid map length of 1595cM. This map is estimated to cover 80% of the genome at an average density of 8.6cM.

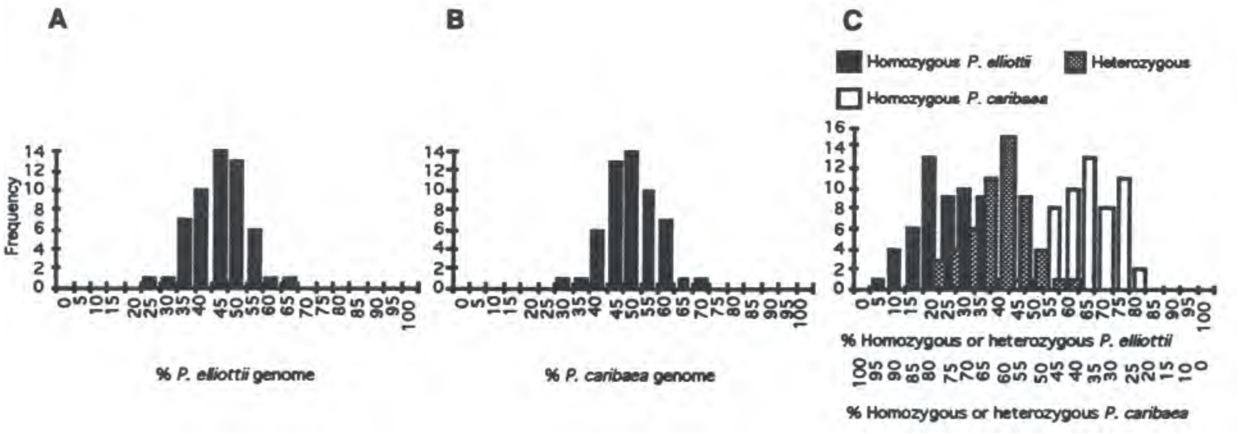
Genotyping of the F2 population and Inference of Fully Classified Genotypes

A total of 139 markers from the megagametophyte derived genetic map of EH4 were used to genotype its F2 progeny at an average density of 11.5cM (Dale et al. 1995). Linkage analysis using fully classified genotype data for the F2 generation revealed marker order throughout the genome to generally remain identical to that of the megagametophyte derived reference map for EH4, except for closely spaced markers. Overall map size differed by 8% between the haploid megagametophyte and diploid F2 populations.

Proportion of each Parental Genome Inherited in the F2 Population

Figure 1, parts a and b, illustrate the frequency distribution for the total proportion of *P. elliottii* and *P. caribaea* genome respectively, inherited in their F2 progeny. On average, the F2 population is comprised of 48.3% *P. elliottii* genome and 51.7% *P. caribaea* genome. This does not differ significantly (P = 0.05) from the expected 1:1 ratio. The minimum proportion of each parental genome inherited by any individual in the F2 was 29.2% and 34.5% for *P. elliottii* and *P. caribaea* respectively. The maximum proportion inherited was 65.5% and 70.7% respectively. In addition, the F2 population conformed to the expected 1:2:1 proportion of alleles heterozygous and homozygous for each parental genotype (Figure 1, part c).

Figure 1. Frequency distributions for. A) the percentage of *P. elliottii* and B) the percentage of *P. caribaea* inherited in their F2 intercross progeny, and C) the percentage of genome heterozygous or homozygous for each parental type.



Identification of Putatively Significant QTL

Simple, unverified results of single locus interval mapping using MapMaker/QTL software detected putative QTL exceeding the minimum LOD threshold of 2.6 for each of the five growth and form traits and four branching characteristics examined. Genomic regions influencing these traits are illustrated in Figure 2a for growth and form traits and in Figure 2b for branching traits. Figure 3 displays the QTL likelihood plots for bark thickness at Tuan, Beerwah and the two sites combined, for one linkage group carrying a region putatively influencing this trait.

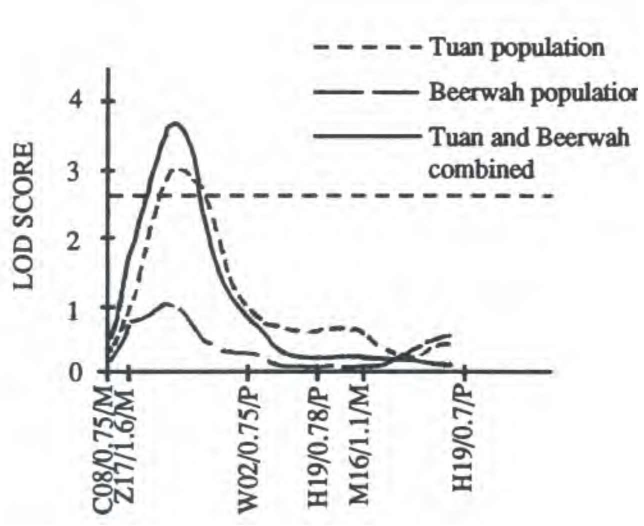
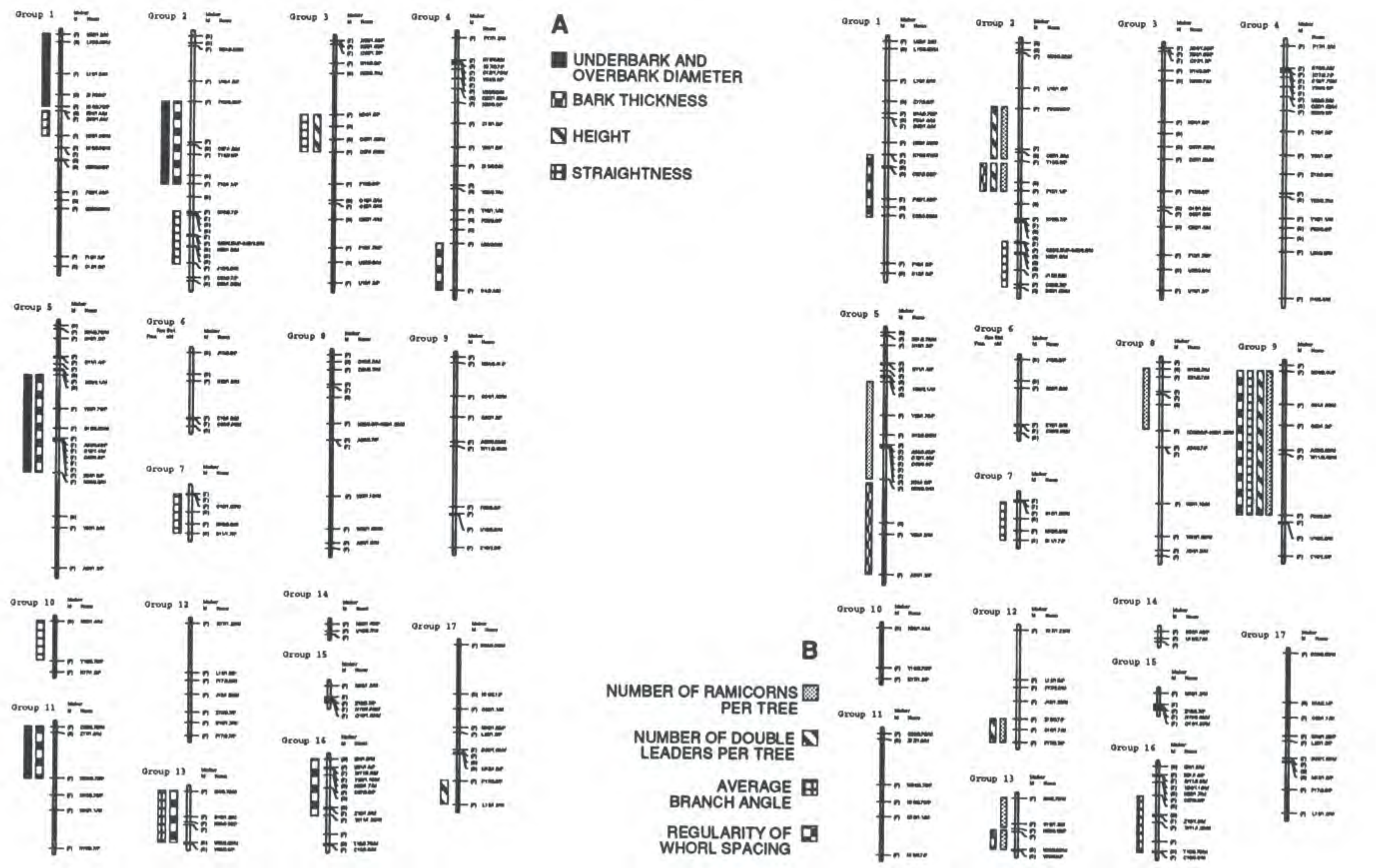


Figure 3. QTL liklihood plots indicating the LOD scores for bark thickness along linkage group 11 of the *P. elliottii x P. caribaea* interspecific hybrid individual, EH4. Likelihood plots are shown for the Tuan and Beerwah sub-populations of the F2 progeny to EH4, and for the the entire F2 family combined across both sites. The horizontal line at LOD 2.6 indicates the threshold for declaration of a QTL.

Using the notation
regions (Y) showing a consistent rise in the LOD score plot for each data set, the following numbers of putative QTL were detected for each trait: average bark thickness, 3(4); diameter at breast height over bark, 2(2); diameter at breast height under bark, 0(4); height, 0(2); straightness, 3(3); number of ramicorns, 6(2); number of double leaders, 4(0); average branch angle, 1(3); and whorl spacing regularity, 3(2).

Figure 2. Putative genomic regions influencing: A) growth and form traits, and B) branching traits in the *P. elliottii x P. caribaea* interspecific hybrid.



Similarity of QTL Between Physiologically Related Traits

Considerable overlap in the set of putative QTL influencing physiologically related traits is evident from Figure 2, parts a and b. Bark thickness, over bark diameter and under bark diameter, all share three putative QTL. Similarly, ramicorns and double leaders share four QTL. These two traits also share a putative QTL with branch angle and whorl spacing regularity.

Detailed Analysis of QTL Effects

Cryptic genetic variation not predicted by the phenotype of either parent was evident among branching QTL. The most significant QTL explaining number of ramicorns (LOD =12.2), double leaders (LOD = 78.1), branch angle (LOD = 2.0), and whorl spacing regularity (LOD= 3.6), all co-incided in the same genomic region, and all displayed an underdominant mode of gene action.

Analysis of the Tuan and Beerwah sub-populations for bark thickness indicated four putative QTL expressed in both environments. For each of these QTL, the LOD score for the Tuan and Beerwah sub-populations roughly summed to that for the combined data set. In contrast, the remaining three QTL identified for bark thickness were expressed in only one environment, and their LOD scores for the combined data set were less than those for either Tuan and/or Beerwah independently.

Three of the putative QTL for bark thickness displayed an additive mode of gene action, this trait being increased by *P. caribaea* alleles for one locus, and *P. elliottii* alleles for the other two. A dominant mode of action was displayed by two other QTL, bark thickness being increased by *P. caribaea* alleles at one locus, and *P. elliottii* alleles at the other. The remaining two QTL both displayed an underdominant mode of gene action, with the heterozygous genotype having thinner bark than the homozygote for either parent.

DISCUSSION

Inference of Fully Classified Genotypes from Dominant RAPD Data

With the availability of a megagametophyte derived reference map, this study has demonstrated that the limitations imposed by the dominant nature of RAPDs may be overcome to permit this technically simple and relatively cost effective marker system to be efficiently applied to the genotyping and analysis of diploid F2 populations. In terms of map resolution, dominant RAPD markers of alternating phase will provide genotype information equivalent to a co-dominant marker map of 62.5% the marker density. The average spacing of 11.5cM between RAPD markers in the present map thus provides a level of genotype precision slightly greater than an equivalent co-dominant marker map of 18.4cM average marker density. This precision is in the order suggested by Lander and Botstein (1989) for detection of QTL, and is unlikely to limit the precision of QTL location given the small population size (Darvasi et al. 1993).

Proportions of each Parental Genome Inherited in the F2 Population

Although both average genome and genotype composition in the selfed progeny of EH4 corresponded to the expected 1:1 and 1:2:1 Mendelian ratios, considerable variation was found in the proportion of parental genome inherited by specific individuals. The minimum composition of *P. elliottii* genome in any F2 individual was just 29.2%. Similarly, the minimum composition of *P. caribaea* genome for any individual was 34.5%. Both these extreme individuals thus have genetic compositions not greatly removed from that expected for an average backcross individual.

The results for both average genome composition and genome composition of extreme individuals correspond very closely with similar studies in tomato (Paterson et al. 1991b). As suggested by Paterson and co-workers, the broad variation found for proportion of each parental genome may provide an opportunity for marker assisted selection (MAS) in F2 and backcross hybrid families, both inbred and outbred. The range of parental genome combinations possible in F2 and backcross populations may potentially produce a proportion of individuals which transgress the performance of Fl hybrids. F2 and backcross hybrids are not presently favoured for operational plantation establishment due to the greater variability they exhibit relative to Fl hybrids (Garth Nikles, pers. comm.). However, the capacity to genotypically characterize F2, backcross and later generation hybrids, and to select genetically superior individuals, may change this perspective. Through clonal propagation of superior genotypes or transgressive segregants (De Vicente and Tanksley 1993), both the additive and nonadditive components of genetic variation inherent in such individuals would be captured. This strategy would also serve to speed the operational deployment of superior germplasm. As genetic markers become a more integral part of forest tree breeding programs, consideration should thus be given to breeding populations showing wide variability, in contrast to the more traditional goal of breeding for uniformity.

Identification of Valid QTL in Small Populations

Numerous putative QTL may be identified by 'black box' application of MapMaker/QTL software, but validation of these and rejection of spurious QTL is necessary to achieve a realistic picture of QTL architecture. This is particularly so for small population sizes (Knapp et al. 1992), and is likely to remain a persistent problem in the application of QTL analysis to forest trees where existing pedigrees, often limited in number and designed for conventional tree breeding purposes, must necessarily be employed. Given the small population size involved in this study, only QTL of large effect will be statistically significant, even though real QTL below the statistically significant threshold may be segregating. Conversely, small population size may result in detection of spurious QTL and biased estimates of QTL effects (Knapp et al. 1992).

Using the results for bark thickness to address this problem, it is evident that there is a consistent trend in the LOD curve between independent populations for some putative QTL. The putative QTL for bark thickness on linkage group 11 provides a suitable example (Figure 3). This putative QTL has a LOD of 1.04 in the Beerwah sub-population and 2.98 in the Tuan sub-population. Although the Beerwah sub-population is not significant, the probability that this same peak occurs in both independent data sets by random chance is $10^{1.04}$ x 10^{198} = 104.02, equivalent to a LOD score of 4.02, a statistically significant value given the appropriate LOD threshold for declaration of a QTL in the present species is 2.6.

Extending this proposition to other QTL for bark thickness, it becomes possible to construct a model for the architecture of this trait composed of environmentally stable, statistically significant and non-significant QTL, plus statistically significant, environment specific QTL. The former are characterized by supporting trends in the data from independent sub-populations, while the latter are specific results from a particular sub-population, unsupported by any other data. This model is illustrated in Figure 4.

In employing the model for bark thickness QTL presented in Figure 4, marker assisted breeding might potentially pursue the alternative strategies of selection for broadly adapted individuals, or selection of individuals adapted to specific sites (Burdon 1977). In the former case, one might select individuals carrying the appropriate alleles for QTL 3, 4, 5 and 6. In the latter, one might select for individuals carrying the same alleles as previously, but in addition for the Tuan site, the appropriate allele of QTL 1.

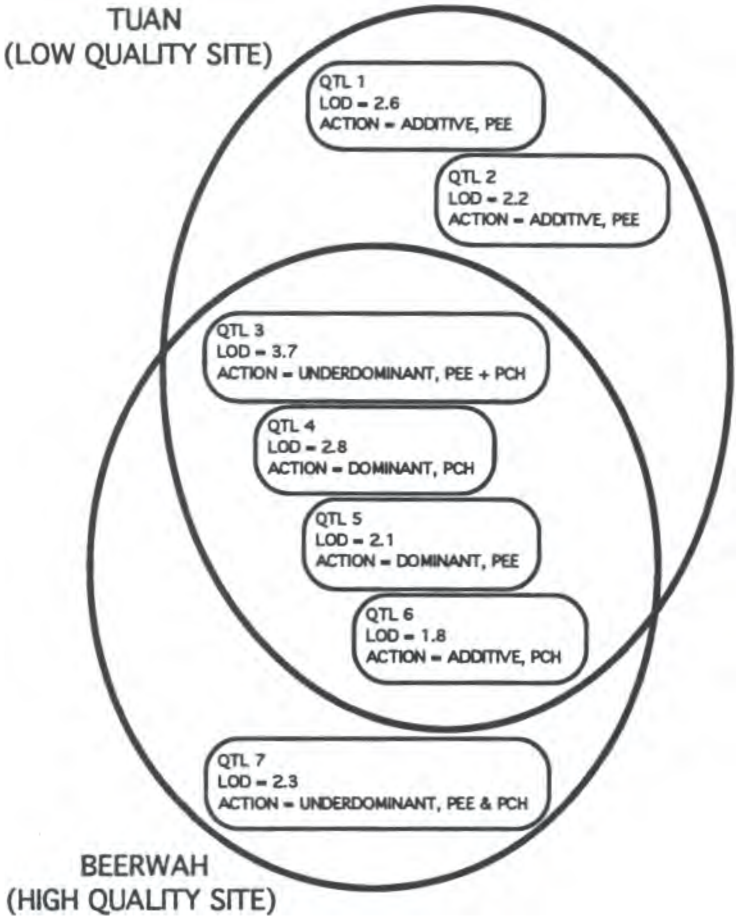


Figure 4. Model for the architecture of bark thickness QTL. This model comprises environmentally stable loci expressed at both Tuan and Beerwah, and evironment specific loci for both sites. For environment stable QTL, LOD score values below 2.6 are considered strongly indicative of putative QTL, since a consistent peak occurs in the likelihood plot for both independent sub-populations (a low probability event by chance alone). In addition, for environment stable QTL, the direction of allelic substitution effect and mode of gene action remain the same for both sub-populations and the combined population. For environment specific QTL, LOD score values over 2.6 are considered significant, but LOD score values below 2.6 provide weak, unsupported evidence for the presence of a QTL.

Similarity of QTL Among Physiologically Related Traits

Commonality among QTL controlling physiologically related traits may also add support to their validity. Bark and wood cells are each laid down in the stem of a tree by bidirectional differentiation of the cambium. An overlap of three QTL between bark thickness, overbark and underbark diameter (Figure 2a) is, therefore, not unexpected from a physiological perspective. These QTL comprise three of the four putatively identified above as environmentally stable QTL for bark thickness on the basis of consistency between sub-populations.

Similarly, QTL for branching characteristics display considerable commonality (Figure 2b). Measurements on numbers of ramicorns and double leaders, average branch angle and regularity of whorl spacing were all physically independent. Yet there exists commonality of loci between ramicorns and double leaders (4) ramicorns, double leaders and whorl spacing regularity (2) and ramicorns, double leaders, whorl spacing regularity and branch angle (1). Of these four branching traits, ramicorns and double leaders each violate the assumptions of normality in their trait distributions, and equal variance between alternate genotype classes (Weller 1992). This has led to inflated LOD scores of 12 and 78 respectively. In isolation, little confidence could be given to the QTL results for ramicorns and double leaders. However, commonality of QTL between related traits adds substantial weight to the validity of these results.

The results for growth and branching QTL appear to indicate strong genetic relationships between physiologically related traits. This alone could have valuable application in selection of trees for conventional breeding programs. For example, ramicorns are rare on trees at Tuan, but common at Beerwah, while double leaders are common at Tuan and absent from Beerwah. However, these two traits share four putative QTL, each of which displays a consistent mode and direction of gene effect between sub-populations. Hence, it is possible that ramicoms and double leaders may be different manifestations of a similar genotype under different environmental influences. This may also explain low heritability estimates in the order of 0.04 to 0.08 reported for double leaders or 'forking' in *Pinus* species (Cotterill and Dean 1990). A practical application of this result in tree breeding may be to consider ramicorns and double leaders as a single trait, and give equal weight to selection against these. Further, as branch angle and whorl spacing regularity appear genetically related, these traits could also be factored into a selection index. Trees with irregularly spaced whorls and steeply angled branches, may give rise to progeny with an unacceptably frequent occurrence of ramicorns or double leaders under altered environmental conditions.

Mode of QTL Effects

For the range of the growth, form and branching traits examined, observed QTL effects included full dominance, additivity, overdominance and underdominance, with both parental alleles and their heterozygous combination variously increasing the value of the traits. This complex mode of cryptic genetic effects, where phenotype of the parental species is not a clear indicator of their progeny's performance, has been reported for a range of traits in other species (De Vicente and Tanksley 1993; Paterson et al. 1991a). Nor is the variety in mode of gene effects unusual. In summarizing the results of six studies, Beckmann (1991), found reports of QTL effects ranging from additivity, through dominance to overdominance.

Interestingly, each of the four QTL for bark thickness identified in Figure 4 as environmentally stable, display a consistent mode of gene action in each sub-population and the combined data set. This consistency is observed when each QTL is analyzed individually, and as a multilocus model. In contrast, the effects of bark thickness QTL identified as environment specific display varying modes of gene action between sub-populations. This result may provide an additional indicator to the validity of both occurrence and mode of effect for putative environmentally stable QTL.

The occurrence of cryptic variation in genotype effects appears to be inversely related to heritability (Paterson et al. 1991a). For such traits, the capacity of MAS to guide selection of parents and control allelic combinations created in their progeny, should prove more efficient in breeding than conventional phenotype based selection (Lande and Thompson 1990), particularly in view of the long generation intervals typical for most forest trees.

CONCLUSIONS

1. Dominant RAPD markers can be used to efficiently provide co-dominant genotype information in F2 populations of gymnosperms.

2. Molecular marker characterization of the broad distribution of parental genome composition in F2, backcross and other advanced generation populations holds significant potential for identification and clonal propagation of superior genotypes, and for introgression of desirable traits from related species.

3. Restricted population sizes characteristic of mature tree breeding families may not necessarily be a limitation to discovery of environmentally stable QTL, and initial application of MAS to forest trees.

4. Similarity of QTL among physiologically related traits may provide guidance for conventional phenotypic selection, particularly for traits strongly influenced by environment.

5. Guided breeding using molecular markers has the capacity to identify and exploit valuable alleles with effects not predicted by their parental phenotype, potentially increasing the efficiency of breeding and rate of genetic gain, particularly for traits of low heritability.

ACKNOWLEDGEMENTS

The authors wish to thank the Forest Service, Queensland Department of Primary Industries, and in particular Garth Nikles, for the production and provision of experimental material. The co-operation of Grant Daggard, Haley Vogel and Chris Gates in development of RAPD protocols and initial mapping work was invaluable. The assistance of Mer y Shepherd in DNA isolation, and of Lyn Parkin, Lara Jefferson, Hong Duong and Jo Walsh in running subsequent RAPD reactions is also gratefully appreciated, *as* was the computing assistance of Derek Harrison and Phil Goodman. We express thanks to Jose Chaparro for valuable discussions and Dave O'Malley for constructive comments on the manuscript. This work was supported by funding from the Australian Rural Industries and Research Development Corporation, Tasman Forestry Ltd., Griffith University, the Queensland Forest Service, and ForBio Pty. Ltd. The principal author was supported throughout this study by a Forestry Postgraduate Research Award from the Australian Commonwealth Department of Primary Industries and Energy.

LITERATURE CITED

Beckmann, J. S. 1991. Genomic genetics and plant genetic improvement, pp. 201-229 in Gene-Mapping Techniques and Applications, Chapt. 10, edited by L. B. Schook, H. A. Lewin and D. G. McLaren. Marcel Dekker, Inc., New York.

Burdon, R. D. 1977. Genetic correlation as a concept for studying genotype-environment interaction in forest tree breeding. Silvae Genet. 26: 168-175.

Cotterill, P. P., and C. A. Dean, 1990 Successful Tree Breeding with Index Selection. CSIRO Publications, Melbourne.

Dale, G. T., G. E. Daggard and R. D. Teasdale. 1995. Genetic analysis of the *Pinus elliottii* x *Pinus caribaea* interspecific hybrid using RAPD markers. I. Linkage mapping with haploid megagametophytes. Theor. Appl. Genet. (manuscript in preparation for TAG).

Dale, G. T., and R. D. Teasdale, 1995 Genetic analysis of the *Pinus elliottii* x *Pinus caribaea* interspecific hybrid using RAPD markers. II. Development of dominant RAPD markers for full genotype classification in a diploid F2 population. Genetics (manuscript in preparation for Genetics).

Darvasi, A., A. Weinreb, V. Minke, J. I. Weller and M. Soller. 1993. Detecting marker-QTL linkage and estimating QTL gene effect and map location using a saturated genetic map. Genetics 134: 943-951.

De Vicente, M. C., and S. D. Tanksley. 1993. QTL analysis of transgressive segregation in an interspecific tomato cross. Genetics 134: 585-596.

Haines, R. J., and S. M. Walker, 1993 Mass vegetative propagation of slash x Caribbean pine: operational status and research directions, pp. 488 in Proceedings of the 22nd Southern Forest Tree Improvement Conference, Atlanta, Georgia, 14-17 June, 1993.

Knapp, S. J., W. C. Bridges and B. H. Liu. 1992. Mapping quantitative trait loci using nonsimultaneous and simultaneous estimators and hypothesis tests, pp. 209-237 in Plant Genomes: Methods for Genetic and Physical Mapping, edited by J. S. Beckmann and T. C. Osborn. Kluwer Academic Publishers, Netherlands.

Lande, R., and R. Thompson. *1990. Efficiency of* marker-assisted selection in the improvement of quantitative traits. Genetics 124: 743-756.

Lander, E. S., and D. Botstein. 1989. Mapping mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121: 185-199.

Lincoln, S., M. Daly and E. Lander. 1992. Mapping genes controlling quantitative traits with MapMaker/QTL 1.1. Whitehead Institute Technical report, No. 2nd edition.

Nikles, D. G. and R. S. Newton. 1991. Correlations of breeding values in pure and hybrid populations of hoop pine and some southern pines in Queensland and relevance to breeding strategies, p192-196 in Proceedings of the 11th Meeting RWGI of the Australian Forestry Council, Coonawarra, South Australia.

Paterson, A. H., S. Damon, J. D. Hewitt, D. Zamir, H. D. Rabinowitch et al. 1991a. Mendelian factors underlying quantitative traits in tomato: comparison across species, generations, and environments. Genetics 127: 181-197.

Paterson, A. H., E. S. Lander, J. D. Hewitt, S. Peterson, S. E. Lincoln et al. 1988. Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. Nature 335: 721-726.

Paterson, A. H., S. D. Tanksley and M. E. Sorrells. 1991b. DNA markers in plant improvement. Advances in Agronomy 46: 39-90.

Proctor, L., A. Rafalski, S. Tingey, S. Lincoln and E. Lander, 1990 MapMaker Macintosh, V1.0. E. I. duPont de Nemours and Company, Wilmington.

Soller, M. 1991. Mapping quantitative trait loci affecting traits of economic importance in animal populations using molecular markers, pp. 21-49 in Gene-Mapping Techniques and Applications, Chapt. 2, edited by L. B. Schook, A. L. Lewin and D. G. McLaren. Marcel Dekker, Inc., New York.

Weller, J. I. 1992. Statistical methodologies for mapping and analysis of quantitative trait loci, pp. 181-207 in Plant Genomes: Methods of Genetic and Physical Mapping, edited by J. S. Beckmann and T. C. Osborn. Kluwer Academic Publishers, Netherlands.

Young, N. D., and S. D. Tanksley. 1991. Hypergene, *V0.92.1.* University of Minnesota and Cornell University, Minnesota and Ithaca.