

GENETIC MAPPING OF WOOD PROPERTIES IN *PINUS ELLIOTTII* VAR. *ELLIOTTII* X *P. CARIBAEA* VAR. *HONDURENSIS* HYBRIDS

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Abstract:--The ability to select clones of uniform wood properties to meet specific end use requirements is viewed as a key element for increasing profitability of exotic pine plantations in Queensland. A genetic mapping project has been initiated to investigate wood properties assessed in 11 year old F₁s. This will lead to a better understanding of the genetic architecture of wood traits and evaluation of the potential for marker-aided selection in the development of improved planting stock. Microsatellite and AFLP markers as well as wood properties will be mapped using a pseudotestcross strategy. This will allow co-segregation analysis between markers and those loci controlling wood traits which are heterozygous in the pure species parents and is most effective where parents are unselected for wood properties. By analysing half and full sib progeny sharing a common parent it is hoped QTL of good hybridising ability can be identified. Plantings over multiple sites will allow investigation of QTL stability across environments. The strategy and recent progress will be outlined.

Keywords: *Pinus elliotii*, *Pinus caribaea* var. *hondurensis*, hybrid, wood properties, genetic mapping

INTRODUCTION

Exotic *Pinus* spp plantations comprise around seventy percent of a total 177 000 ha plantation estate in Queensland. Of this, currently seventeen percent is an interspecific hybrid between *P. elliotii* Engelm var. *elliotii* Little and Dorman (PEE) and *P. caribaea* Morelet var. *hondurensis* Barrett and Golfari (PCH). This hybrid is the taxon of choice due to its superiority in growth and form across a range of sites in Queensland (Toon *et al.* 1996; Powell and Nikles 1996; Rockwood *et al.* 1991). It is deployed as seedlings or as cuttings from superior control pollinated families. Currently, a small proportion of F₁ planting material comprises superior clones with the objective of a fully clonal production system by 2002 (Walker *et al.* 1996).

Wood from the F₁ is utilised primarily for structural timbers, veneer and plywood products (Nikles 1996). Key wood criteria include: basic density, latewood percentage, extractive content, mean grain spirality and uniformity at the tree and plantation levels (Rockwood *et al.* 1991; Harding 1996). Several studies of wood properties in the parental taxa have indicated there is considerable variation within each group for most key properties (Harding *et al.* 1991; Allen 1985). Generally PEE has a higher basic density and higher latewood percentage but lower extractives than PCH (Rockwood *et al.* 1991). Similarly, in the F₁ there is a large amount of variability in mean basic density, latewood percentage and mean grain spirality amongst F₁ clones or F₁ families (Harding 1996). Little comparative data amongst the 3 taxa is available. Preliminary data suggests, however, that the sawn graded recovery is greater for hybrid than pure PEE, and that the F₁ is intermediate for density and latewood but similar for other properties (Rockwood *et al.* 1991 and references within). Importantly, stability of wood properties across a range of locations was evident both at the family and taxon levels (Rockwood *et al.* 1991).

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Information on the heritabilities of wood properties for parental species indicates important wood properties have moderate to high values. A study of PCH assessed on 2 sites at age 10 yr indicated individual narrow sense heritabilities for basic density and latewood percentage were 0.65 ± 0.20 and 0.55 ± 0.19 respectively and 0.29 ± 0.14 for mean radial ring width (Harding et al. 1991). Individual tree narrow sense heritability estimates for basic density and latewood percentage were similar in PEE 0.69 and 0.78 for trees aged 15 yr (Allen 1985). Hence, there would appear to be both significant variability in current parental and hybrid populations and opportunity for significant gains in breeding for wood properties. Furthermore, wood properties are poorly related to growth and form (Allen 1985) suggesting improvement should be possible without adverse effects on other characters.

Despite apparently favourable criteria for genetic improvement and their importance to the end products, breeding for wood properties has not been emphasised in the *Pinus* breeding programs in Queensland. The breeding programs for both PEE and PCH in Queensland currently focus on volume and form for the identification of parents with the highest predicted ability to produce superior hybrid families (Walker et al. 1996; Toon et al. 1996). Individuals with very poor wood characteristics, however, may be eliminated from further testing. The relatively low priority of wood properties has largely been due to the expense in assaying for wood properties and the delay before reliable assessments are obtainable. For example, where within tree uniformity is a target for improvement, detailed densitometry studies are required which are costly for the collection and analysis of samples (Harding 1996; Grattapaglia 1997).

In this paper, we outline a genetic mapping approach to investigate the genetics of wood properties in the PEE x PCH F₁ hybrid. The objective is to improve understanding of the genetic factors contributing to variation in the hybrid and evaluate the potential for marker-aided selection (MAS) within the context of a clonal forestry program. Here we outline the strategy and methods proposed for this project and report on results to date. We discuss the rationale for this strategy and comment on potential opportunities and outcomes.

METHODS

Population and field trial design

The mapping experiment is based on 12 full-sib hybrid families which are a subset of 144 F₁ families generated from the intermating of 12 select PCH parents with 12 select PEE parents established by Queensland Forestry Research Institute (QFRI) in 4 locations in 1987 (QFRI Expt. 674). Parents were selected on the basis of good growth and form but not on wood properties and have been used to generate a number of commercially important F₁ hybrid clones. The trial was laid out in randomised complete blocks at each of 4 sites with taxa separated into blocks (Powell and Nikles 1996). Within each taxon, families were planted as 2 tree non-contiguous plots, randomly located within a 72 tree block. One F₁ family was sampled for both foliage and wood cores at a rate of 23-31 trees per site at four sites (a total of 107 trees) whereas the remaining 11 families, sharing a common paternal parent, were sampled at a rate of 3-11 trees at each of 2 sites (a further 119 trees).

GENETIC MARKERS

Microsatellites

Enriched microsatellite libraries for a PEE (2EE1-166) and a PCH (1CH6-29) individual were generated using the approach of Edwards et al. 1995. A supplemental screening using DIG labelled

oligonucleotides was used to increase the efficiency of detecting clones containing microsatellites due to the low efficiency of standard enrichment processes when applied to the conifer genome (see Poster 10 this conference).

In addition, microsatellite loci from *P. radiata* and *P. taeda* are being investigated for transfer to the hybrid (Smith and Devey 1994; Fisher et al. 1996; Echt et al. 1996; Echt and Burns 1999; Minihan et al. 1999). PCR conditions are reported with the respective sources and products and were sized using fluorescently labelled primers on a ABI 310 Genetic Analyser (Perkin Elmer, Foster City, CA).

AFLP

To increase genome coverage, AFLP markers are being developed for mapping in these families. The approach taken follows that used in other large genome organisms, such as *P. taeda* (Pers. Comm. David Remington) *P. edulis* (Travis et al. 1998) and *Alstroemeria* (Han et al. 1999) using a 2 bp pre-selection and 7 bp selective amplification. Restriction, ligation reactions were prepared as per "AFLP protocol manual" (Perkin Elmer). Pre-selection and selective amplification were as described by Vos et al. 1995 using primer sequences kindly provided by D. Remington. Selective primers with the core *EcoRI* adaptor sequence were fluorescently labelled and products sized using a ABI 310 Genetic Analyser.

DNA preparations

Genomic DNA from foliage of the 2 parents and 89 F, progeny was prepared according to the method of Scott et al. 1997. DNA was also extracted from megagametophytes of open pollinated seed from each parent. Megagametophyte tissue was frozen in liquid N₂ and crushed with a nail or in BioPulverizer Tubes (BIO 101 La Jolla, CA USA) by vortexing prior to DNA extraction with a DNeasy™ Kit (QIAGEN Valencia, CA USA).

Wood properties analysis

Bark to bark 12mm diametral cores were taken at 1.3 m above ground from F, progeny using a motorised increment corer in 1998. Cores will be assessed for basic density and latewood percentages using direct scanning x-ray densitometry. This is ongoing work undertaken by the QFRI Wood Quality Improvement Laboratories, Brisbane.

Linkage mapping and QTL analysis

A genetic linkage for the individuals 2EE1-166 and 1CH1-63 will be generated from 94 F, progeny using a pseudotestcross strategy using microsatellite and AFLP markers (Grattapaglia and Sederoff 1994). In this approach, heterozygous marker loci present in one parent but absent from the other parent are informative for mapping. Genetic maps will be generated using Mapmaker software (Lander et al. 1987) following the procedure described by Grattapaglia and Sederoff 1994.

Cosegregation analysis of wood properties characteristics and markers will be conducted after transferring a framework map of evenly spaced markers to all 12 year old progeny. Marker-trait linkages may be identified using several approaches including single marker regression (Weller 1992), interval mapping (Lander and Botstein 1989) or composite interval mapping (Zeng 1994; Jiang and Zeng 1995) in packages such as QTL Cartographer (Basten et al. 1999).

RESULTS

MICROSATELLITES

Development of microsatellites in PEE and PCH

An initial screen of 70 clones from an enriched library for the PCH individual and 20 from the PEE individuals resulted in a poor recovery of clones containing microsatellites. This was thought, in part, to be due to the application of standard enrichment techniques to the large genome of conifers. Nonetheless, a total of 3 clones from the PEE library and 5 from the PCH library contained microsatellites. Five primer pairs were designed, 3 of which successfully amplified microsatellite regions. Microsatellites have been tested on a number of individuals from each parental species and a sample of 6 progeny from a 2EE1-102 X 1CH1-63 cross. Two loci tested to date are polymorphic between the parents of this cross with marker allele segregating in a Mendelian manner.

Following a secondary screening of the library using DIG labelled oligonucleotides, 43/43 clones contained microsatellites (See Poster10 for further details). Further microsatellites markers will be developed from these sequences.

Transfer of microsatellites from other *Pinus* sp.

Twenty six microsatellite primer pairs from *P. taeda*, nine from *P. radiata* and six from *P. strobus* have been examined for transferability to PEE and PCH (Table 1). Of the 5 loci from *P. strobus* 3 amplified in PEE and PCH, however, none were polymorphic within and between a sample of 2 individuals from each taxon. Five out of eight markers from *P. radiata* amplified in PEE and PCH. Of these, 3 were polymorphic amongst the six individuals from each group. 18 microsatellite markers developed from a genomic library of *P. taeda* were tested in PEE and PCH, 12 amplified, 11 appear to amplify microsatellite regions and are polymorphic amongst PEE or PCH or between PEE and PCH. Primer pairs for eight microsatellite loci identified in EST from *P. taeda* were also tested in PEE and PCH. Seven primer pairs apparently amplify microsatellite regions in PEE and PCH. Allele diversity between the two sources of sequences was not significantly different (average of 1.9 and 2.3 for EST and genomic sources respectively).

AFLP

Polymorphism in AFLP profiles is a consequence of mismatching between the selective bases of the selective amplification primers and template genomic fragments. Commonly, for plants with regular genome sizes (5×10^8 to 6×10^9 bp) 3 base extensions on each primer are used to reduce profile complexity and detect sequence polymorphism. Conifers with larger genomes, typically around 2-3 times of the above upper limit, have required increased selectivity to reduce profile complexity (D Remington Pers. Comm.; Cato 1999). Preliminary, evaluation of 4 primer combinations with 3+4 bp selections has revealed a low level of polymorphism amongst several individuals from PEE and PCH (1-2 polymorphisms) QFRI breeding populations and amongst 4 *P. radiata* individuals (av 5), each from a different provenance (Samples kindly provided by Southern Tree Breeding Association).

Table 1. Allele diversity in *P. caribaea* and *P. elliotii* for Microsatellite loci derived from several conifers

Locus	Species of Origin	Repeat type	Expected size	<i>P. caribaea</i> ¹			<i>P. elliotii</i> ¹		
				Amp.2	n	alleles	Amp.	n	alleles
scu Pch 37	<i>P. caribaea</i> (CT)		166	162	1	1	162	1	1
scu Pch 48	<i>P. caribaea</i> (CA)		246	254	5	3	248	3	2
scu Pee 25	<i>P. elliotii</i> (TG)		150	150	5	4	120	3	2
PtTX 2008	<i>P. taeda</i> di		307	305	4	2	305	4	2
PtTX 2037	<i>P. taeda</i> di		177	163	4	3	159	4	4
PtTX 2123	<i>P. taeda</i> tri		202	201	4	2	201	4	2
PtTX 2128	<i>P. taeda</i> di?		245	238	4	1	238	4	1
PtTX 2142	<i>P. taeda</i> di		262	238	4		238	4	2
PtTX 2146	<i>P. taeda</i>		180		4			4	
PtTX 2164	<i>P. taeda</i> tri		252		4			4	-
PtTX 3011	<i>P. taeda</i> di		186	174	4	2	158	4	1
PtTX 3013	<i>P. taeda</i> tri		134	132	4	3	141	4	3
PtTX 3017	<i>P. taeda</i>		212		4		n	4	-
PtTX 3019	<i>P. taeda</i> di		223	221	4	4	208	4	1
PtTX 3020	<i>P. taeda</i>		211	195	4		186	4	
PtTX 3025	<i>P. taeda</i>		266	268	4	2	268	4	2
PtTX 3026	<i>P. taeda</i>		344		4			4	
PtTX 3030	<i>P. taeda</i>		287		4			4	-
PtTX 3032	<i>P. taeda</i>		335		4			4	-
PtTX 3034	<i>P. taeda</i> di		207	207	4	2	207	4	7
PtTX 3037	<i>P. taeda</i> di?		144		4		129	4	1
RPT 1	<i>P. taeda</i> (ATA)		125	124	4	3	124	4	2
RPT 11	<i>P. taeda</i> (CAT)		213	214	4	2	214	4	3
RPT 13	<i>P. taeda</i> (CTG)		277	273	4	2	276	4	2
RPT 15	<i>P. taeda</i> (GTG) (TGG) (TGG) (T)		246		4			4	
RPT 20	<i>P. taeda</i> (CAG)		259	254	4	1	254	4	2
RPT 5	<i>P. taeda</i> (CAA) (CAA) T (AAC)		197	195	4		195	4	1
RPT 8	<i>P. taeda</i> (GCG)		196	192	4		195	4	2
RPT 9	<i>P. taeda</i> (A) (TG) (GAG) (CAG) (GCA) (GCA)		289	282	4	3	258	4	2
NZPR1 (locus 2)	<i>P. radiata</i> (AG) n		139	132	5	5	136	5	5
NZPR2	<i>P. radiata</i> (AG) n		162	162	1		90	1	
NZPR4	<i>P. radiata</i> (AG) n		146				n	1	
NZPR5 (Locus 2)	<i>P. radiata</i> (AG) n		114	82	5	3	82	5	3
NZPR6	<i>P. radiata</i> (AG) n		198	182	5	2	190	4	3
NZPR7	<i>P. radiata</i> (AG) n		149	79	5	5	115	5	7
PR-4.6	<i>P. radiata</i> (AC)		206-222				n	1	
PR-9.3	<i>P. radiata</i> (AC)		92-108		1		n	1	
RPS105	<i>P. strobus</i> (AC)		151	142	2		143	2	1
RPS150	<i>P. strobus</i> (CAG)		248	246	2		246	2	1
RPS160	<i>P. strobus</i> (ACAG)		246		2		n	2	
RPS3	<i>P. strobus</i> (AC)		287		2			2	
RPS61	<i>P. strobus</i> (AC)		195	175	2	1	175	2	1

1. Select individuals from a population used in operational plantations. Samples kindly provided by QFRI.
2. Amplification: n=no or size of the allele closest to the expected size from original genotype or size range where exact allele size has not been determined.

DISCUSSION

Why map wood properties in an interspecific F₂?

There are several general reasons why wood properties are a suitable target for genetic improvement. Firstly, the close link between the economic value of forest products and wood properties suggest that managers want improvement to be quickly translated into more profitable plantations (Harding 1996). Several genetic considerations also suggest significant gains can be achieved rapidly. Moderate to high heritabilities of many key wood criteria and hence potentially fewer loci to manage in improvement programs may translate into more rapid gains (Grattapaglia 1997). Additionally, the high degree of variation and unselected nature of many breeding populations also suggest there will be scope for rapid gain. Furthermore, the low correlations between wood properties and other traits suggest gains should be possible with minimal adverse effects on other traits (Allen 1985; Zobel and Jett 1996). These reasons apply equally to conventional improvement as well as molecular improvement programs.

Genetic mapping and subsequent marker aided selection (MAS) may offer several additional opportunities for the improvement of wood properties. Although molecular assays are currently too expensive for many tree improvement programs, the technology is rapidly becoming more accessible. In time, it may be possible to replace costly wood properties assays. However, it is the opportunity for early assessments of wood properties, before they can be phenotypically evaluated, where perhaps MAS has its greatest merit. This benefit may be further increased in the context of a clonal forestry program (see below). Finally, genetic mapping should provide the greatest detail of genetic architecture on wood properties, ie the number of loci, relative magnitude of effects and epistasis (Strauss *et al.* 1992). This may lead to better estimates of gain from MAS and conventional breeding and optimisation of breeding strategies (O'Malley and McKeand 1994).

Opportunities for MAS of wood properties in the context of a clonal forestry program

Increased uniformity of wood properties at a plantation level should be achievable in a clonal forestry program. Plantation uniformity may be improved through plantings of clonal blocks while also allowing a diversity of wood properties between blocks to meet specific market requirements (Harding 1996). Furthermore, increased within tree uniformity may be realised quickly from a clonal program as desired genotypes can rapidly be deployed. The economics of growing plantations could be very positively impacted if reduced rotation ages were possible without sacrificing wood quality. Shorter rotations rapidly increase the proportion of juvenile wood in the harvest and it is this juvenile wood in pines which it is critical to improve to achieve acceptable wood quality.

This process could be expedited further by application of MAS (Dale and Chaparro 1996; Grattapaglia 1997). Within family selection at an early age is an important application for marker-aided selection in forestry (Dale and Chaparro 1996; Grattapaglia 1997; O'Malley and McKeand 1994). Once linkage relationships are identified between favourable QTL allele and marker alleles, these should hold for several generations in progeny from these parents (O'Malley and McKeand 1994). These associations may be utilised by recreating the family, so that outstanding progeny are able to be selected on marker genotype at the seedling stage. This is important for traits such as wood properties, which need to be assessed late in the rotation or where maturation of propagating material is a problem (Dale and Chaparro 1996). Further benefit from the application of MAS in a clonal forestry context should arise from the flow of selected progeny into clonal testing programs where it may be possible, for example, to test fewer clones yet increase the value of material exiting the testing program.

Favourable marker-QTL linkages may also be utilised across families sharing a common parent, if QTL of good average effect have been identified linked to markers (O'Malley and McKeand 1994, Plomion and Durel 1996). In our study we hope to identify QTL of average effect for a paternal parent from an analysis of a half sib array with 12 maternal parents. It is necessary to evaluate a half-sib array as QTL detected in single full-sib families can not be understood in terms of average effect (O'Malley and McKeand 1994). Markers linked to such QTL should also be useful for MAS in unmapped crosses sharing the same common parent.

Further benefit from MAS may be obtainable for population improvement by retaining individuals with good average effect QTL in the breeding populations (O'Malley and McKeand 1994). Population improvement should also benefit from the more rapid turnover of generations achievable with MAS, in the context of recurrent selection in elite populations (Grattapaglia 1997).

The uniformity of wood properties of a clone may also be affected by the environment. It will be important to know whether QTL are stable across environments or reactive. This project will examine wood properties across several sites. Data from each site may be treated as a separate trait but tested for QTL simultaneously in a model in composite interval mapping allowing estimates of genotype by environment interactions (Jiang and Zeng 1995).

Mapping strategy

The diverse interspecific F₁ hybrid, is the primary base material for improvement in the QFRI clonal forestry program. Therefore, it is genetic loci which control variation in the F₁, that is of major interest. This variability is a consequence of the heterozygosity of the parents (Bradshaw and Grattapaglia 1994). Inbred-like F₂ mapping strategies in wide crosses between individuals of outcrossing forest species explore, largely, the genetic variation as a consequence of loci which are treated as fixed for different alleles between the grandparents (Williams 1998, Bradshaw and Grattapaglia 1994). This variation may not be relevant to the F₁ generation. Such strategies have been applied to investigations of loci responsible for heterosis in poplar hybrids (Bradshaw and Grattapaglia 1994; Bradshaw and Stettler 1995). Utilisation of MAS in a three generation pedigree would require re-evaluation of the recurrent hybrid breeding program to place a greater emphasis on F₁ or backcross generations (Bradshaw and Stettler 1995). Similarly, it remains largely unknown whether loci important for economic traits in pure species will be the same as those effective in an interspecific hybrid (Bradshaw and Grattapaglia 1994). This is an issue of considerable importance for tree breeders working with hybrids (Powell and Nikles 1996) and may be the subject of future investigations by comparing QTL influencing wood properties in the hybrid with QTL identified in crosses with the parental species.

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