

Congruence in QTL for adventitious rooting in *Pinus elliottii* × *Pinus caribaea* hybrids resolves between and within-species effects

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Received: 12 September 2005 / Accepted: 26 February 2006
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Abstract Targeting between-species effects for improvement in synthetic hybrid populations derived from outcrossing parental tree species may be one way to increase the efficacy and predictability of hybrid breeding. We present a comparative analysis of the quantitative trait loci (QTL) which resolved between from within-species effects for adventitious rooting in two populations of hybrids between *Pinus*

elliottii and *P. caribaea*, an outbred F₁ ($n=287$) and an inbred-like F₂ family ($n=357$). Most small to moderate effect QTL (each explaining 2–5% of phenotypic variation, PV) were congruent (3 out of 4 QTL in each family) and therefore considered within-species effects as they segregated in both families. A single large effect QTL (40% PV) was detected uniquely in the F₂ family and assumed to be due to a between-species effect, resulting from a genetic locus with contrasting alleles in each parental species. Oligogenic as opposed to polygenic architecture was supported in both families (60% and 20% PV explained by 4 QTL in the F₂ and F₁ respectively). The importance of adventitious rooting for adaptation to survive water-logged environments was thought in part to explain oligogenic architecture of what is believed to be a complex trait controlled by many hundreds of genes.

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Keywords Marker-aided selection · Rooting
percentage · Stem cuttings · Synthetic hybrids

Introduction

Species hybrids of *Populus*, *Eucalypt*, *Salix*, *Larix* and *Pinus* dominate plantation forestry programs in a small number of situations throughout the world where they outperform parental taxa because of hybrid complementarity or F₁ heterosis. Although breeding for hybrids often proceeds successfully with

little knowledge of the genetic basis of hybrid complementarity or heterosis, a knowledge of genetic architecture, i.e. the numbers of genes, the relative magnitudes of effects, gene action and interactions, nonetheless, would be valuable for predicting genetic gains and successful hybrid combinations, as well as optimizing breeding and marker-aided selection (MAS) strategies (Kerr et al. 2004b; Vaillancourt et al. 1995; Bradshaw and Grattapaglia 1994).

Population improvement of conifers and other forest trees generally focuses on quantitative traits and relies largely on gains from additive components of genetic variation (Zobel and Talbert 1984; Eldridge et al. 1994). The importance of dominance and other interaction effects in heterosis indicates that breeding strategies based on additive genetic variance alone are unlikely to be optimal for many, if not most, interspecific hybrids (Bradshaw and Grattapaglia 1994; Dieters et al. 1997; Rieseberg et al. 2000).

Targeting between-species effects for selection may be one way to improve the efficacy and predictability of breeding with synthetic hybrid populations. Synthetic hybrids are new breeding populations based on two or more species (or breeds) that offer a heterotic advantage and/or a combination of favourable characters from the foundation species. Simulation studies of synthetic hybrid populations of some trees have found that they offer the highest amounts of genetic gain per year over a range of genetic structures (Kerr et al. 2004a). Depending on the generation, genetic variances of hybrid families derived from parental populations of outcrossing species will be affected by the loci that segregate due to the allelic variation both between and within individuals in the parental species populations (i.e. within-species effects), as well as effects that are fixed for contrasting alleles between the species (between-species effects). The genetic variance within an F_1 family will be affected by segregation at genetic loci that are heterozygous in the individual parents (within-species effects) (Bradshaw and Grattapaglia 1994). The contribution of within-species effects to variation amongst F_1 hybrids is of practical importance to breeders in species where hybrid are selected for clonal deployment (Bradshaw and Grattapaglia 1994). It is variation due to between-species effects that should be the basis of predictable F_1 heterosis because it is due to fixed differences between parental species (Bradshaw and

Stettler 1995; Forbes et al. 2004). In advanced generation hybrid breeding of trees it may be advantageous to increase the frequency and re-fix alleles from the favourable species in breeding populations.

Quantitative trait loci (QTL) analysis has the potential to resolve the sources of variance into their individual underlying genetic loci, and to parameterise gene action and interaction effects (Stuber 1989). From a large number of QTL studies of interspecific F_1 hybrids of forest trees, there is emerging consensus that within-species effects are typically small to moderate in size (Individual QTL explaining 1–5% of phenotypic variation (PV) (reviewed in Shepherd and Jones 2005). Between-species effects detected in interspecific F_2 plant hybrids on the other hand, are typically much larger (often from 20 to 50% PV but as much as 88%), because they may result from loci that are divergent in each species because of adaptation, speciation or domestication (Bradshaw and Stettler 1995; Bradshaw et al. 1995; Paterson et al. 1991; Doebley and Stec 1991). Few studies have provided a comparative analysis of these different sources of genetic variance in hybrid plant populations and little is known about the gene action and relative importance of these effects in different hybrid generations. A comparative analysis of QTL contributing to variance in both an F_1 and F_2 may be one way of resolving within and between-species effects, because QTL that are congruent in F_1 and F_2 populations should correspond to within-species effects, whereas loci that are unique to the F_2 population are potential between-species effects.

We are interested in genetic improvement of vegetative propagation characters of the hybrid between *Pinus elliottii* Engelm var. *elliottii* Little and Dorman and *P. caribaea* Morelet var. *hondurensis* Barrett and Golfari. Plantation forestry in subtropical and tropical Australia is primarily based on exotic pines, and the hybrid is the most suitable taxon for the majority of plantation estate on the coastal areas of central and south-east Queensland (Haines 2000). The F_1 hybrid is difficult and expensive to propagate by seed hence production of the hybrid is based on a rooted cutting system (Walker et al. 1996). Since 2002, the annual planting requirements have been supplied from hybrid stock consisting of tested clones. Rooting success of cuttings has a major impact on the cost of production. Production is currently limited by the range of genotypes that can be

deployed because of low rooting rates, and maturation effects that cause a decline in rooting as stock plants age (Shepherd et al. 2005).

Here we report on a comparative analysis of QTL in two unrelated families, an outbred F_1 (herein F_1) and inbred-like F_2 (herein F_2). The F_1 family was typical of those used in the clonal forestry program. The F_2 family was an experimental population, generated from the selfing of an interspecific F_1 , and represented the type of material that may arise from synthetic hybrid breeding population that will be used for future breeding (Brawner et al. 2005). We were interested in resolving between- from within-species effects and characterizing their relative contributions to genetic variances. A high level of congruency in QTL of small-moderate effect size in the two families validated most QTL detected and was believed to identify within-species effects, whereas a single large effect QTL unique to the F_2 was believed to be a between-species effect. QTL parameters for between- and within-species effects were compared and the implications of QTL architecture for breeding and MAS are considered.

Methods

Taxa and populations

This study comprised two hybrid families between *Pinus elliottii* var. *elliottii* Engelm. and *P. caribaea* var. *hondurensis* Morlet (Genus *Pinus* subgenus *Pinus* section *Pinus* subsection *Australes* Louden), (Little and Critchfield 1969) (Table 1). The first family was an F_2 generated by self-pollinating a

select F_1 hybrid individual (eh43) from the interspecific cross between a *P. elliottii* seed parent (1ee1-015) and a *P. caribaea* pollen donor (1ch6-029). The second family, an F_1 , was generated from a controlled-pollination of a select *P. elliottii* (2ee1-102) maternal parent with pollen from a select *P. caribaea* (1ch1-063) (Table 1). Although the species' parents of each cross were not related, they originated from the same natural provenance/region. Both *P. elliottii* individuals originated from bulked seedlots mainly collected from north east Florida and south east Georgia regions (Nikles 1996). The *P. caribaea* parents were both selections from the Mountain Pine Ridge provenance (Belize).

Pinus elliottii (slash pine) occurs in south eastern USA from South Carolina in the north, south to central Florida and west to Louisiana (Little 1971). It habits mainly low lying coastal plains in sandy soils which can remain water logged for long periods during the year (Wakeley 1954). *Pinus caribaea* variety *hondurensis* occurs discontinuously in Central America, from about 18°15' N in parts of the uplands of Quintana Roo (Mexico) to about 12° N in Nicaragua including, Guatemala, Belize, as well as occurring in Honduras and its coastal islands (Farjon and Styles 1997; Dvorak et al. 2000). Despite its discontinuous distribution, varieties of *P. caribaea* have similar levels of isozyme variability to continuously distributed species of outcrossing conifers (Zheng and Ennos 1999). The early Queensland Department of Primary Industries Forestry (QDPIF) breeding populations of *P. caribaea* var. *hondurensis* were largely comprised of material from an upland region of Belize (Mountain Pine Ridge provenance) (Nikles 1996) where it inhabits ridge sites with shallower soils (Greaves 1978). *Pinus elliottii* has

Table 1 Pedigree and rooting percentage parameters for subpopulations in the F_1 and F_2 families

Family	GP1 ^a	GP2	P1	P2	Family (n)	Family rooting percentage range (Min–Max) (%)	Family rooting percentage (mean±SD) (%)	SG (n)	SG low (mean±SD) (%)	SG high (mean±SD) (%)	QTL est. popn (n)
F_2	1ee1-015	1ch6-029	eh43	eh43	357	0–100	56±46	32	0±0	100±0	196
F_1	–	–	2ee1-102	1ch1-063	287	0–100	58±31	32	0±0	100±0	224

^aGP=grandparent; P=parent, SG=selective genotyping population, low and high phenotypic classes for rooting percentage; QTL Est.=QTL estimation; Popn=population

greater wind firmness and responds to flooding by developing adventitious roots more so than either the hybrid or *P. caribaea* (Lewy 1990; Anonymous 1977). The two species do not hybridise naturally because they are allopatric and have asynchronous flowering times. They cross readily artificially but F_1 viability is variable and often low; however, viability is restored in outcrossed F_2 (Richter and Duffield 1951; Nikles 1966; Nikles et al. 1999).

Vegetative propagation and rooting percentage assessments

Production of seedlings, cuttings and nursery management of the genetic material used in this study has been previously reported (Shepherd et al. 2005). In brief, seeds were sown in February 1998 and the seedlings were over wintered in a heated glasshouse, then planted out in the hedge production area of the DPIF nursery at Toolara, Queensland in May 1999. Germination rates were approximately 80% in each family. The total number of ortets (seedlings) planted was 288 and 408 in the F_1 and F_2 families respectively. Cuttings were collected from the 287 and 356 (F_1 and F_2 respectively) surviving, 31 month old ortets in the week commencing 11th September 2000. Sufficient cuttings were taken to give three replicates of four cuttings per clone (i.e. a total of 12 cuttings per clone). Clones were arranged sequentially in trays and in contiguous replicate blocks within the nursery beds. Progress of rooting percentage was monitored by assessing rooting percentage in excess material managed using identical conditions to that of the main trial. Assessment was carried out when approximately 50% of clones in each family had rooted (19 weeks post-setting in January 2001). Rooting percentage was recorded as the proportion of rooted cuttings in each replicate.

A full analysis of genetic determination of rooting in the hybrids is reported by Shepherd et al. (2005). The family mean of the F_1 was higher than the F_2 (Table 1). This was probably due to the effects of inbreeding depression but may have also been due to the different genetic backgrounds of each family. Phenotypic and genetic variances (broad sense heritability) were consistently higher for the F_2 than the F_1 (Shepherd et al. 2005)

Genetic maps for *Pinus*

Microsatellite markers for this study were selected from a sex- and species-averaged consensus map of the individual trees *P. elliottii* var. *elliottii* (2ee1-102) and *P. caribaea* var. *hondurensis* (1ch1-063) with some linkage information inferred from a third map for *P. taeda* (Zhou et al. 2003) (haploid chromosome number=12 for all three taxa). The markers chosen were selected based on their informativeness in the F_1 cross of these trees, marker reproducibility and to achieve a spread of evenly spaced markers along the map. The consensus map consisted of 91 microsatellite markers distributed on the 13 homeologous and three parent specific-linkage groups (Appendix 1). The map covered a total distance of 727.1 cM Kosambi which represents around 43% of *Pinus* genome based on map distance of 1700 cM for *Pinus taeda* (Remington et al. 1999).

Microsatellite markers

Microsatellite markers for the genetic map were transferred from related species. A set of 201 *Pinus* spp. microsatellite markers (Hicks et al. 1998; Fisher et al. 1998; Devey et al. 2001; Smith and Devey 1994; Elsik et al. 2000; Auckland et al. 2001; Zhou et al. 2002; Echt and Burns 1999; Soranzo et al. 1998) were evaluated for transfer to the taxa of interest, *P. elliottii* and *P. caribaea*. One hundred and 20 markers transferred, but 21 were excluded either due to complex banding patterns (9) or did not segregate in parents (13), leaving a set of 97 informative cross-specific primer-pairs which provided 99 loci for linkage analysis. In addition two loci, SCUPEE25 and SCUPCH48 were developed de novo in the parental taxa. They were isolated from microsatellite enriched libraries of the two parents according to the method of (Edwards et al. 1996). The sequences for these primers were [SCUPCH48] 5'CAGGTTGAGGCCACCT-AGA3' and 5'TTAGGCCAAATAATGACAAGAC3'; and [SCUPEE25] 5'GAAAATATTATAAGGGAA-GTTCT3' and 5'GACCGATTAGTATCCTCATA-CA3'. The SCUPEE25 locus contains a (CA)₉ and SCUPCH48 contains a (CA)₁₇ repeat.

Microsatellites were amplified using a PCR buffer containing a final concentration of 20 mM Tris-HCl (pH 8.4), 50 mM KCl (supplied as 10× PCR buffer

(Invitrogen Life Technologies), 2.0 mM MgCl₂, 0.1 mM dNTP (total for 4 dNTPs), 0.02/U/ μl Platinum *Taq* (Invitrogen Life Technologies) and 20 ng of DNA template in a total volume of 11 μl. The cycling parameters were those originally reported with each locus. Thermocycling was carried out on a Perkin Elmer 9700 thermocycler (Applied Biosystems). The cycling conditions for SCUPCH48 were; an initial hold of 2 min at 94°C followed by 35 cycles of 94°C for 0.1 s, 60°C for 30 s, 75°C for 1 min, followed by a final hold at 75°C for 5 min. The cycling conditions for SCUPEE25 were the same except that the annealing temperature was 52°C. Each primer was synthesised with a 5' end fluorescent dye by either Genset Pacific (Lismore, Aust.) or Applied Biosystems. PCR products were size and dye multiplexed post-PCR, for separation by capillary electrophoresis (AB 310 or AB3700 Genetic Analyser; Applied Biosystems). Alternatively, PCR products for some primers-pairs were labelled using R110 dUTP (Applied Biosystems).

Linkage analysis

Mating configurations for each locus were determined from the genotype of the two parents and the 86 F₁ hybrid offspring. Markers were coded for parental or a backcross (hybrid) population for analysis using JoinMap 3.0 (Van Ooijen and Voorrips 2001). Map construction was iterative, with a preliminary round of grouping and ordering to identify possible mis-scored data. The map with lowest chi-square value was used to order loci at each stage using a grouping at a minimum LOD of 3 and locus ordering with a maximum Chi-square ‘‘Jump’’ value of 5 (decrease in Chi-square goodness-of-fit measure that may accompany the addition of locus to an order). In a second round of ordering, markers that could not be phased or had insufficient linkage for ordering were eliminated and the best order re-established. Parental maps were compared for apparent order discrepancies and raw data was re-examined for improbable genotypes (based on the large chi-square values from the ‘‘genotype probabilities’’ tab sheet in the ‘‘Map’’ node in Joinmap). At this stage there were two apparent order discrepancies between the parental maps, each on a separate linkage group. Eliminating 1 or 2 individuals, respectively, from the data set for each linkage group

exhibiting improbable genotypes, resolved each of these apparent discrepancies in paternal map order. A sex- and taxa-averaged consensus map was generated for homeologous linkage groups identified in *P. elliotii* and *P. caribaea* using the Combine-groups function of JoinMap 3.0. Marker grouping and ordering in the two parental maps (*P. elliotii* and *P. caribaea*) was compared to the sex-averaged *P. taeda* map (Map 4) (Zhou et al. 2003). Homeologous linkage groups from the three maps were aligned visually then examined for synteny and collinearity.

Genotyping for the QTL study

DNA extractions

DNA from frozen pine needles or megagametophyte tissue of seed was extracted using a DNeasy 96 plant DNA kit (Qiagen, Valencia, CA) using the frozen tissue protocol with modified described in Shepherd et al. (2002).

Microsatellite genotyping

All microsatellite markers were amplified with a PCR buffer containing a final concentration of 20 mM Tris-HCl (pH 8.4), 50 mM KCl (supplied as 10× PCR buffer (Invitrogen Life Technologies)), 2.0 mM MgCl₂, 0.1 mM dNTP (total for 4 dNTP's), 0.2 μM of each primer, 0.02 U/ul Platinum *Taq* (Invitrogen Life Technologies) and 20 ng of DNA in a total volume of 15 μl. The exceptions to this were: (1) PtTX4055 which was amplified with a final MgCl₂ buffer concentration of 3.3 mM; and (2) genotyping in the F₂ population, used reaction conditions of Devey et al. (2001) for all markers originally identified in *P. radiata*. The PCR cycling parameters used were those reported with the primer-pairs i.e. (Devey et al. 2001; Auckland et al. 2001; Elsik et al. 2000; Fisher et al. 1996, 1998; Echt et al. 1996; Echt and Burns 1999; Smith and Devey 1994). Thermocycling was carried out on a Perkin Elmer 9700 thermocycler (Applied Biosystems). One primer in each pair was synthesised with a 5' end fluorescent dye by either Proligo (Lismore, Australia) or Applied Biosystems (Foster City, CA, USA). PCR products were size and dye multiplexed post PCR, for separation by capillary electrophoresis on an Applied Biosystems 310

Genetic Analyser. Scoring of markers was semi-automated using Genotyper software NT v 3.5 (Applied Biosystems).

Marker-trait associations

A two stage approach was used to detect and characterise marker-trait associations. In the detection stage, a selective genotyping approach and single marker tests (SMT) were used to identify genomic regions associated with adventitious rooting. Interval mapping (IM) and multiple-QTL model (MQM) mapping were used to estimate QTL parameters in the second characterisation stage.

Selective genotyping

A selective genotyping strategy was used to identify markers that showed linkage to adventitious rooting. A selective genotyping population of 32 individuals (the 16 lowest and 16 highest rooting clones in each family) was identified in each family using the rooting percentages for each clone obtained from the nursery trial. A list of markers genotyped in each family is given in Appendix 1. Although genome coverage was similar, the marker sets in both families were not identical, as some markers were uninformative in both families and substitutes were necessary.

Single-marker tests

Tests for independence in contingency tables (2×3 for the F_2 ; $2 \times 4 - 2$ for the F_1) were used to detect marker-trait associations using counts of the clones categorised by genotypic and phenotypic classes. Tests for independence were implemented using the Crosstabs module of SPSS (SPSS Inc. Chicago, Illinois) with Fisher's exact probabilities reported (Also see Appendix 2).

Genotyping a large random sample for unbiased estimates of QTL parameters

Large random samples from both families were genotyped in addition to their selective genotyping populations to allow estimation of QTL parameters. Totals of 224 and 192 individuals were genotyped in the F_1 and F_2 family respectively.

Interval mapping

Interval mapping was conducted using MapQTL v4 (Van Ooijen et al. 2002) to estimate QTL position and magnitude in the large samples from each family. Additive models were tested in the F_1 whereas dominance was fitted in models to test for QTL in the F_2 . Interval mapping was conducted at 5 cM intervals along maps for the linkage groups identified as associated with rooting percentage by single-marker analysis in selective genotyping populations. Permutation tests with 1000 permutations were used to establish experiment-wise significance thresholds (Churchill and Doerge 1994).

To test for multiple QTL per linkage group, Multiple QTL Model (MQM) mapping was conducted using MapQTL. MQM analysis provides additional resolution for testing for multiple QTL by regressing out the effect of other QTL using linked markers as cofactors (Jansen and Stam 1994). Hence, in addition to the IM model tested for each linkage group in each family, both an MQM and restricted MQM (rMQM) analysis were conducted. In MQM, the effects of all other QTL except the test QTL were controlled for, whereas in rMQM, only QTL residing on linkage groups other than the group currently being tested were controlled. Cofactors were selected using the automatic cofactor (backwards elimination) analysis module of MapQTL.

For some loci, in the F_1 , more than one genetic model could be fitted, depending on the mating configuration. Allele substitution effects were tested by contrasting alternative alleles from one species pooled across alleles from the second parent. Backcross models were possible by contrasting genotypes with alternative alleles from one parent in a fixed background for the other parent. Alternative models were tested by ANOVA using the compare means module of SPSS.

Results

A genome screen for QTL using selective genotyping in the F_2 family

A scan of the mapped region of the *Pinus* genome was carried out to identify regions putatively associated with rooting percentage. Fifty-one markers,

distributed across 15 linkage groups, were screened on a selective genotyping population ($n=32$) of 16 high and 16 low rooting clones from the F_2 family (Appendix 1). The average rooting percentages for low and high rooting classes were 0 ± 0 and 100 ± 0 , respectively (Table 1). Eleven markers, clustered on three linkage groups (Pe7, H9 and H10), were significantly associated with rooting percentage (Fisher's exact test point-wise P -value < 0.05) (Table 2 and Fig. 1).

QTL synteny in an independent selective genotyping population from an F_1 family

Fifty-six informative markers were screened on a selective genotyping population ($n=32$) of 16 low and 16 high rooting percentage clones (av. rooting percentage 0 ± 0 and 100 ± 0 respectively) from the F_1 family (Table 1). Eight loci distributed on four linkage groups were significantly associated with rooting percentage (Fisher's exact test point-wise P -value < 0.05) (Tables 3 and 4 and Fig. 1). Six of the eight loci were located on three linkage groups (Pe7, H9 and H10), which were also associated with rooting percentage in the F_2 family. Three of these markers, PtTX3024 (group H9), PtTX3105 (group Pe7) and

PtXT4228 (group H10) were associated with rooting percentage in both families. Two loci (PtXT3123 and APC11) were located on a fourth group (H11), which was not associated with rooting in the F_2 (Fig. 1). Hence correspondence of position was found in two families for QTL on three linkage groups Pe7, H9 and H10 and a fourth linkage group H11 was identified in the F_1 only.

Multiple contrasts were possible for two loci with a MIC4 or MIC3 mating configuration in the F_1 , (see methods and Appendix 2). For example at PtTX3034 (a marker with a MIC4 configuration), three contrasts were testable, an MIC4/G (contrasting genotypic means for all four genotypic classes), and MIC4/E and MIC4/C, tests for substitution effects for the alleles from the *P. elliotii* and *P. caribaea* parents respectively (constructed by pooling genotypic means on alternate alleles from one parent). The MIC/G test value was significant (P -value=0.012), whereas the test contrasting *P. caribaea* alleles (MIC/C) was highly significant (P -value=0.003), but the test contrasting *P. elliotii* alleles (MIC/E) was non-significant (P -value=0.713). This suggested most of the variance attributable to this locus was due to the substitution effect of *P. caribaea* alleles, rather than as a consequence of *P. elliotii* heterozygosity. In

Table 2 Single-marker tests for rooting percentage in a selective genotyping population ($n=32$) from the F_2 family

Linkage group ^a	Marker	P ^b	GP ^b	GP	Offspring counts (low rooting %)			Offspring counts (high rooting %)			Fisher's exact test P -value
					EE ^c	Het.	CC	EE	Het.	CC	
		eh43	1ee2-015	1ch6-029							
Pe7	PtTX2126	290/328	290/328	290/290	8	7	1	1	13	2	0.020
H9	PR111	125/184	123/184	125/125	7	7	0	1	7	7	0.003
H9	PtTX3049	297/300	297/297	297/300	10	6	0	0	8	8	0.000
H9	PtTX4214 ^d	245/null	245/245	242/null	16	0	–	10	6	–	0.018
H9	PtTX4042	126/140	140/142	126/126	10	6	0	0	10	6	0.000
H9	PtTX3107	152/162	152/162	152/165	10	6	0	1	10	5	0.001
H9	PtTX4147	185/194	194/194	185/191	10	4	2	1	11	4	0.003
H9	PtTX3024	281/297	263/281	270/297	9	4	2	2	12	2	0.011
H10	PtTX3105	177/189	188/189	173/177	0	9	7	6	6	4	0.033
H10	PtTX4228	162/168	168/null	158/162	0	10	6	6	5	5	0.017
H10	RPTest09 ^e	249/268	249/254	268/273	1	12	3	8	6	2	0.023

^aLinkage group and marker order are from a consensus linkage map (Appendix 1). H="Homeologous" linkage group constructed from synteny across three species of *Australes* subsection of *Pinus*. Pe = *P. elliotii* linkage group with no homeologue identified in the other species. Markers used in this study are listed in Appendix 1

^bP=Parental and GP=grandparental microsatellite phenotypes expressed in base pair allele sizes

^cOffspring genotypes were designated as follows; CC=homozygote for *P. caribaea* allele of hybrid parent; Het.=heterozygote; EE=homozygote for *P. elliotii* allele

^dPtTX4214 was analysed as a dominant marker

^eNote: PtTX2146 is the same genetic locus as RPTest09 See Williams et al. (2001)

Fig. 1 Congruence of rooting percentage QTL in a *P. elliotii* × *P. caribaea* F₂ and an F₁ family detected by single marker, interval mapping and MQM analyses. Four genomic regions (linkage groups Pe7, H9, H10 and H11) from a *P. elliotii*/*P.*

caribaea consensus map are shown (map distance cM Kosambi). QTL synteny was declared when at least one SMT per linkage group was significant in both families (*P*-value <0.05 per individual test). SMTs were carried out on a selective genotyping population (*n*=32) in each family. Markers which were significantly (individual test *P*-value <0.05) associated with rooting percentage by SMTs in the F₂ are underlined. Those significant in the F₁ are italicised and those that were significant in both families are both underlined and italicised. Interval mapping was conducted on a larger sample (*n*=196 and 224; F₂ and F₁ respectively) to estimate QTL parameters. Interval mapping (IM) LOD curves are indicated on the right of each group. A LOD threshold of 2.2 and 2.6 was required for experiment-wide significance of α = <0.05 in the F₂ and F₁ respectively. MQM and rMQM mapping were also performed to test for multiple QTL per linkage group. The two QTL peaks detected in the F₂ family on group H9 are indicated with a “1” and “2” respectively. Markers used as cofactors have an asterisk appended to their names

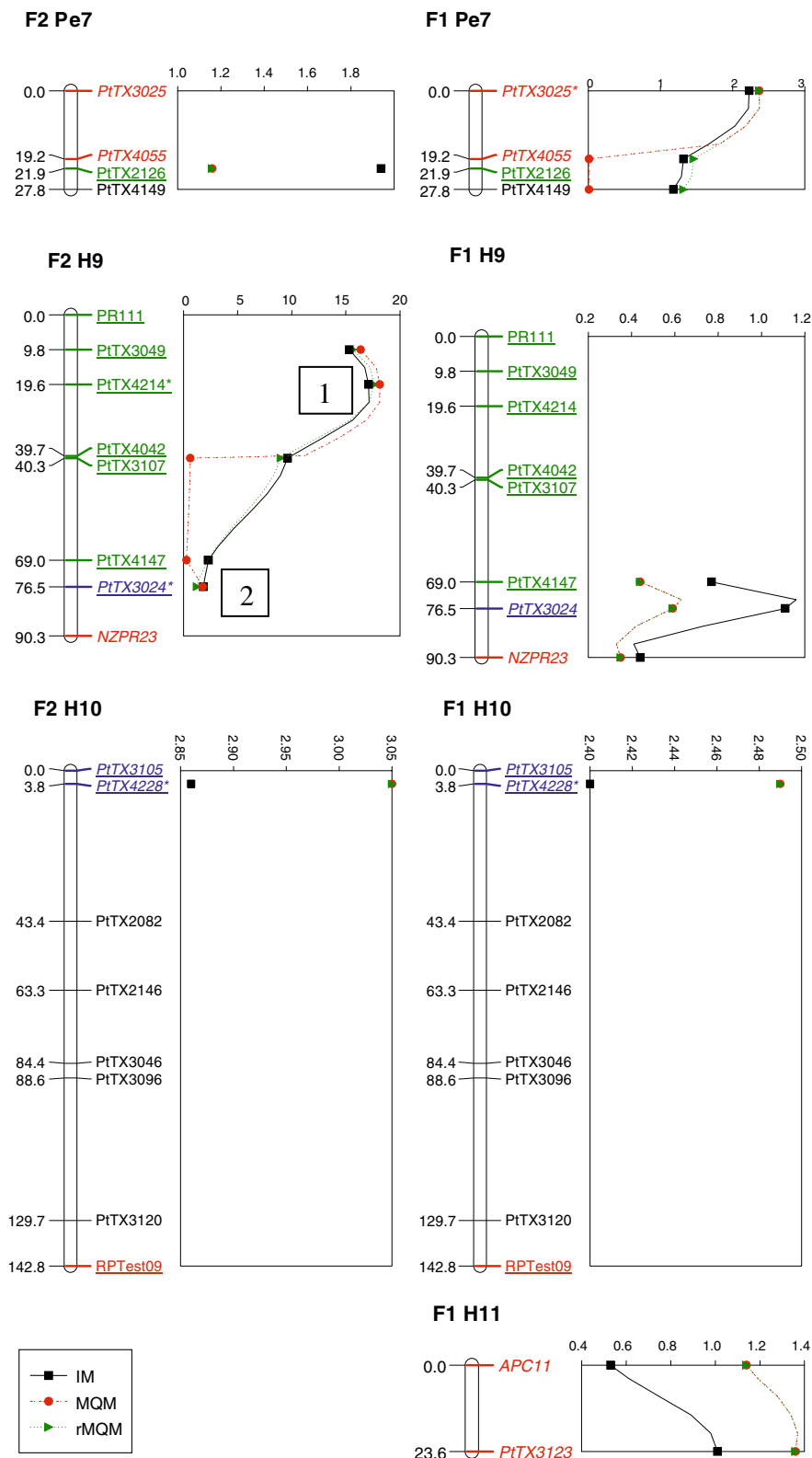


Table 3 SMTs for rooting percentage in a selective genotyping population ($n=32$) from the F_1 family

Linkage group ^a	Marker	<i>P. elliotii</i> parental genotype	<i>P. caribaea</i> parental genotype	Low rooting % offspring class (counts)				High rooting % offspring class (counts)				Fisher's exact test ^c
				E1/E2 ^b	C1/C2	E1/C1	E1/C2	E2/C1	E2/C2	E1/C1	E1/C2	
Pe7	PtTX3025	263/264	263/263	0	–	15	–	10	–	6	–	0.000
Pe7	PtTX4055	208/209	208/208	2	–	13	–	9	–	7	–	0.023
H9	PtTX3024	248/306	275/296	0	7	0	7	4	2	4	6	0.012
H9	NZPR23	null/null	113/null	12	3	–	–	5	11	–	–	0.011
H10	PtTX3105	186/null	null/null	14	–	1	–	7	–	9	–	0.006
H10	PtTX4228	166/168	152/152	1	–	14	–	9	–	7	–	0.006
H11	PtTX3123	209/null	null/null	11	–	3	–	5	–	11	–	0.014
H11	APC11	157/165	181/null	7	3	1	4	3	1	4	8	0.169

Contrasts for mating types with four genotypes or backcross configurations (MIC/G or BC respectively)

^aSee Table 2

^b*P. elliotii* and *P. caribaea* alleles were designated E1, E2 and C1, C2 respectively

^c*P*-values reported. Several contrasts were possible for some mating configurations. See Appendix 2 for methods

contrast, a similar analysis of locus APC11 (group H11), also with a MIC4 mating configuration, indicated heterozygosity in *P. elliotii* parental was the main source of variation at this QTL.

QTL parameter estimation from a large sample in the F_2

QTL position and magnitude of effect was estimated using interval mapping. An additional 160 individuals (to give a total of 192 including the 32 individuals

from the selective genotyping population) were randomly selected for genotyping with a sub-set of 7 of 11 loci previously associated with rooting percentage in the selective genotyping population of the F_2 . All markers, except PtTX3024 and PtTX2126, were also significantly associated with rooting percentage in the larger sample (experiment-wide *P*-value $<0.05=$ LOD 2.2) (Table 5 and Fig. 1). A large LOD peak (LOD 17.1) occurred on group H9 between PtTX4214 and PtTX3049 and explained 42.5% of the PV (Fig. 1). LOD peaks located on the other two linkage groups

Table 4 SMTs for rooting percentage in a selective genotyping population ($n=32$) from an F_1 family ($n=32$)

Linkage group ^a	Marker	<i>P. elliotii</i> parental genotype	<i>P. caribaea</i> parental genotype	MIC/E				MIC/C				Fisher's exact test ^c	
				Low		High		Low		High		MIC/E	MIC/C
				E1	E2	E1	E2	C1	C2	C1	C2		
Pe7	PtTX3025	263/264	263/263									na	na
Pe7	PtTX4055	208/209	208/208									na	na
H9	PtTX3024	248/306	275/296	7	7	6	10	0	14	8	8	0.713	0.003
H9	NZPR23	null/null	113/null									na	na
H10	PtTX3105	186/null	null/null									na	na
H10	PtTX4228	166/168	152/152									na	na
H11	PtTX3123	209/null	null/null									na	na
H11	APC11	157/165	181/null	10	5	4	12	8	7	7	9	0.032	0.724

Allele substitution contrasts for *P. elliotii* and *P. caribaea* parent (MIC/E and MIC/C respectively)

^aSee Table 2

^b*P. elliotii* and *P. caribaea* alleles were designated E1, E2 and C1, C2 respectively

^c*P*-values reported. Several contrasts were possible for some mating configurations. See Appendix 2 for methods

Table 5 QTL parameters for rooting percentage in the F₂ (n=192) estimated by interval mapping^a

Linkage group	Locus	Position (cM K) ^b	QTL							
			LOD	EE	Het.	CC	% expl.	Add ^c	Dom ^d	D/A
Pe7	PtTX2126	21.9	1.94	0.37	0.51	0.62	4.6	-0.12	0.02	-0.16
H9	PtTX3049	9.8	15.33	0.22	0.54	0.77	32.30	-0.27	0.04	-0.15
H9	–	14.8	16.76	0.18	0.57	0.81	42.50	-0.32	0.08	-0.25
H9	PtTX4214	19.6	17.1	0.15	0.61	0.81	49.30	-0.33	0.13	-0.40
H9	PtTX3107	40.3	9.63	0.26	0.52	0.73	21.70	-0.24	0.02	-0.10
H9	PtTX4147	69.0	2.31	0.36	0.52	0.58	5.40	-0.11	0.06	-0.51
H9	PtTX3024	76.5	1.83	0.40	0.56	0.45	4.3	-0.03	0.14	-5.14
H10	PtTX4228	3.8	2.86	0.68	0.49	0.40	6.80	0.14	-0.05	-0.37

^aInterval mapping results are reported at each tested marker and any intervening peaks. The experiment-wise significance thresholds were determined by permutation testing. LOD 1.8, 2.2 and 2.8 for P -value ≤ 0.1 , 0.05 and 0.01, respectively

^bLocation of marker or QTL peak along a linkage group in centiMorgan (cM) Kosambi (K). See Fig. 1

^cAdd=additive effect $(EE-CC)/2$ (Van Ooijen et al. 2002)

^dDom=dominance effect= $Het-(EE+CC)/2$

(Pe7 and H10) were comparatively smaller, explaining 4.6% and 6.8% of variation respectively (Table 5). Additive and dominance components were estimable in this F₂ family. Dominance was relatively unimportant at QTL peaks, usually ranging from 10 to 50% of the additive component (Table 5). The exception was at PtTX3024 where the QTL effect tended toward overdominance where dominance was around 5.5 times the additive effect. Favourable alleles for rooting percentage originated from *P. caribaea* at two QTL (H9 and Pe7) and from *P. elliottii* at one QTL on H10 (Table 5).

QTL parameter estimation from a large sample in the F₁

To estimate QTL parameters in the F₁, a further 192 randomly selected individuals were genotyped for a total of nine loci distributed on the four linkage groups previously associated with rooting percentage QTL in the selective genotyping population of this family. This included seven of the eight loci previously associated with rooting percentage in the F₂ family (PtTX3105 was excluded due to reproducibility difficulties) plus two linked but non-significantly associated markers (PtTX4149 and PtTX4147, Table 6). No QTL peak was significant by interval mapping at the experiment-wide LOD threshold of 2.6 (P -value ≤ 0.05); however, two QTL peaks were significant at a suggestive level (P -value ≤ 0.1 ,

Table 6). One peak occurred on Pe7 at PtTX3025 (LOD 2.3) and a second peak occurred at PtTX4228 on H10 (LOD 2.40). At the two loci (PtTX3024 and APC11) with MIC4 mating configurations (See Appendix 2 for mating configurations), where it was possible to compare allele substitution effects of the two parents, in both cases the effect of substituting *P. caribaea* was about 3–6 times larger than substituting *P. elliottii* alleles (Table 6). This suggested a greater variance of allele effects in *P. caribaea* than *P. elliottii* at these two QTL.

Testing for multiple QTL per linkage group

To test for multiple QTL per linkage group, MQM mapping was used to fit two models in addition to the IM model used in the above analysis. An MQM model and a restricted MQM rMQM (restricted MQM) model were examined for each linkage group in both families. Comparison of the LOD surface plots for the three models for group H9 indicated that most likely two QTL segregated in the F₂, but only one QTL in the F₁ family (Fig. 1). Two QTL were indicated in the F₂ because there were two distinct peaks in the MQM mapping plot. As MQM mapping controlled for the presence of multiple QTL on the same linkage group, it suggested the large QTL (Peak 1) (peaking between PtTX 3049 and PtTX 4214) masked the presence of the smaller QTL (Peak 2) near PtTX 3024 in the IM and rMQM analyses.

Table 6 QTL parameters for rooting percentage in an F₁ family ($n=224$) estimated by interval mapping^a

Linkage group	Locus	Mating conf.	Position (cM K) ^b	QTL							
				LOD	E1/C1	E1/C2	E2/C1	E2/C2	% expl.	PCH ^d	PEE
Pe7	PtTX3025	BCF	0.0	2.23	0.51	0.51	0.65	0.65	4.6	0.00	0.28
Pe7	–	–	5.0	2.22	0.51	0.51	0.66	0.66	5.3	0.00	0.3
Pe7	PtTX4055	BCF	19.2	1.32	0.52	0.52	0.63	0.63	2.7	0.00	0.22
Pe7	PtTX4149	BCF	27.8	1.18	0.53	0.52	0.62	0.62	2.5	0.01	0.19
H9	PtTX4147	BCM	69.0	0.77	0.63	0.54	0.61	0.54	1.6	0.16	0.02
H9	–	–	74	1.16	0.66	0.53	0.61	0.54	2.9	0.20	0.04
H9 ^c	PtTX3024	MIC4	76.5	1.11	0.65	0.53	0.61	0.54	2.3	0.19	0.03
H9	NZPR23	BCM	90.3	0.44	0.60	0.56	0.51	0.61	1.5	0.06	0.04
H10	PtTX4228	BCF	3.8	2.40	0.66	0.66	0.52	0.52	4.9	0.00	0.28
H11	APC11	MIC4	0.0	0.53	0.61	0.52	0.59	0.58	1.1	0.10	0.04
H11	PtTX3123	BCF	23.6	1.01	0.66	0.45	0.59	0.60	5.8	0.20	0.08

^aInterval mapping results are reported for each marker and also any intervening peaks. The experiment-wise significance thresholds determined by permutation testing were LOD 2.2, 2.6 and 3.4 for $P < 0.1$, 0.05 and 0.01 respectively

^bSee Table 5

^cPtTX3024 was significantly associated with rooting percentage when the two extreme phenotypic classes were contrasted (P -value=0.047) (see text)

^dAllele substitution effects. e.g. $PCH = [(E1/C1 + E2/C1) - (E1/C2 + E2/C2)]$

Different gene action at each of these QTL also supported the hypothesis that there were two QTL on group H9 in the F₂ (Table 5). A relatively high dominance/additive ratio (5) was found at PtTX3024 compared with all other markers on group H9 (0.03–0.44). The congruence of the LOD surface plots for all three models in the F₁ indicated that most likely there was only a single peak that corresponded closely in position with the second smaller QTL in the F₂.

MQM analysis also suggested that peak positions for QTL on Pe7 may not correspond between the F₁ and F₂ (Fig. 1). The MQM LOD surface plot located the QTL peak at PtTX3025 in the F₁ whereas the peak occurred at PtTX2126 in the F₂. Precise comparisons of peak positions within this linkage group was confounded by different sets of segregating markers in each family, hence not too much emphasis could be placed on this result.

Discussion

Oligogenic QTL architecture of adventitious rooting in hybrid pines

The genetic architecture of adventitious rooting in pines is unknown but it is assumed to involve the

action of many genes. From studies of the model plant *Arabidopsis* and other well studied plants, it is known that genetic control of rooting is complex and probably involves many hundreds of genes (Casson and Lindsey 2003). A fundamental question for complex traits involving many genes is whether each gene has a uniformly small effect (polygenic) or whether the distribution of gene effects is such that there are a small number of genes of major effect explaining a large proportion of the variation (oligogenic). In this study of adventitious rooting in two unrelated hybrid pine families, we observed QTL architecture that was oligogenic in the F₂ family (four QTL were identified accounting for a total of 68% of the phenotypic variance) and tending toward oligogenic architecture in the F₁ (four QTL accounting for ~18% phenotypic variance). Because of limited experimental power and map coverage our estimates for the numbers of loci involved were likely to be conservative and some effects may have gone undetected. Nonetheless, the evidence favoured an oligogenic as opposed to a strictly polygenic model for adventitious rooting in the hybrid pines.

An adaptive role for adventitious rooting in the natural populations of these *Pinus* sp. may partially explain oligogenic architecture. There is growing empirical evidence from QTL studies that shows much of the variation in adaptive traits can be

explained by a few major effects (Orr and Coyne 1992; Bradshaw and Stettler 1995; Paterson et al. 1991). Although the mechanism by which the large effects contributing to variance of adaptive characters arise remains equivocal (Ridley 2004), a recent review of the population genetics of adaptive traits indicates exponential-like distributions of effect sizes are observed because the leading factors fixed early during adaptation are always large (Orr 1998). Hence, although evolution is generally thought to proceed by small changes, large effects could be common in plant evolution where species invade new niches or other situations where a species has moved from an adaptive optima e.g. during domestication (Doebley and Stec 1993; Orr 1998).

An adaptive role for adventitious root development was suggested because it is an important response for plant survival under stress or flooding (De Klerk et al. 1999). The two parental species in our study, *P. caribaea* and *P. elliotii*, inhabit non-overlapping native environments, upland ridge sites in Belize, South America and water-logged sandy soils in Georgia and northern Florida (USA) respectively. Experience from growing both parental taxa as exotics in Queensland, has shown *P. caribaea* is intolerant of water logging compared to *P. elliotii* (Lewty 1990). *P. elliotii* possesses morphological features that are associated with flood tolerance, producing adventitious roots and other physiological responses to flooding when grown under flooded conditions in the glasshouse (Lewty 1990). Our working hypothesis was that there could be strong selection for adventitious rooting in *P. elliotii* as it is adapted to water-logged environments whereas there was likely to have been less selection in *P. caribaea*.

QTL congruence validates QTL and distinguishes within-species effects

A comparative analysis of QTL was carried out to (1) validate QTL (2) investigate QTL stability across genetic backgrounds and different levels of inbreeding, and (3) test whether between and within-species effects could be distinguished. There was a high level of congruence of QTL, with three QTL in each family mapping to corresponding positions in the maps of the two families, as well as a corresponding absence of QTL on the remaining linkage groups. Congruent QTL tended to have small to moderate effects

(2–5%). Congruency of QTL was important for validation of QTL. Correspondence of QTL location, at a rate greater than expected by chance in independent populations, is strong evidence that genes influencing the phenotype locate to that particular genomic region (Paterson et al. 1995; Doebley and Stec 1993).

Congruence of QTL was expected for within-species effects, where one or both species' parents for both families were heterozygous. These loci also segregated in the F₂ because the F₁ parent has an allele from each parental species and therefore were highly likely to differ in effect. In addition to segregating for within-species effects, the F₂ also segregated for those loci fixed between parental species (between-species effects). One putative between-species effect was identified in this study (QTL H9 Peak 1), a large effect QTL explaining ~50% PV were identified uniquely in the F₂. It was around 10 times the size of the average within-species effect in this family. The approach taken, (i.e. comparing QTL in the F₁ and F₂), has tentatively allowed differentiation of between and within-species effects and examination of the relative magnitudes of these effects. The majority of QTL (congruent QTL—within-species effects) were stably expressed across the two genetic backgrounds and in the two populations with different inbreeding coefficients. These effects were considerably smaller than the less frequent (1 in 4) between-species effects.

Factors promoting QTL congruence

In this study, QTL congruence may have been promoted by oligogenic architecture; low experimental power to detect small effects; factors that increase effect size (hence QTL are more reliably detected in multiple populations) and, factors promoting genetic diversity in one or both parental species populations. Congruence of QTL would be rare under a polygenic model. Congruency should decrease with an increasing number of effects, as the probability that a QTL and marker loci for each effect will segregate in multiple families will rapidly decrease. Congruency should also decrease with effect size, as smaller effects will be harder to detect reproducibly. Low experimental power to detect small effect QTL would have also contributed to congruence as many small effects may have been effectively hidden from detection, reducing the number of QTL detected in

each family and confining the investigation to relatively large effect QTL that may be detected more reliably. Effectively we may not have been studying congruence in the many small effects as they may have been below the threshold of detection.

Inter-genomic interaction may have been a third factor contributing to QTL congruence in this study by inflating genetic variances, increasing effect size and therefore the reproducibility of detection in different populations. Over dominance at one of the shared QTL loci (H9 Peak 2 at PtTX3024) in the F₂ may be evidence of inter-genomic interaction. Post-zygotic isolation mechanisms in plants are attributed to both genic and chromosomal structural incompatibilities (Rieseberg 2001). Genetic mechanisms are thought to be most often the cause of hybrid inferiority (viability or fertility) acting via incompatible gene combinations (negative epistasis) to reduce introgression at hybrid fitness loci (Burke and Arnold 2001). Poor fitness or heterotic performance of heterozygous (EC genotype) compared to parental types (EE and CC) may result from hybrid incompatibility. As H9 Peak 2 was a congruent QTL, segregating in both families, hybrid incompatibility may be contributing to genetic variance in both generations. Field performance also suggests there could be compatibility issues with this hybrid. The *P. elliotii* × *P. caribaea* hybrid is an intersectional cross and exhibits markedly reduced and variable seed germination (0.2–43.6%) in F₁ hybrids compared with its parents (Slee 1970). High rates of aberrant morphology and mortality are also found in the F₁ compared with parental taxa or outcrossed second generation hybrids (Powell 2000). Trees may appear normal in the nursery and survive 3–4 years in the field before showing signs of collapse and eventually mortality.

Diversity at a locus in at least one parental species should also promote QTL congruence. As the F₂ should segregate for almost all loci (only those fixed for the same allele in both species will not segregate), allelic diversity in the parental populations was probably more limiting than heterozygosity of the F₁ parent for QTL congruence in this study because it determined whether or not a locus segregated in the F₁. QTL congruence suggested that high diversity was maintained at most loci for rooting in at least one parental species. Our data further indicated that variance at QTL was greater for *P. caribaea* than

P. elliotii. The effect of substituting alleles at within-species effects in the F₁ for *P. caribaea* tended to be 2–6 times larger than substitution of *P. elliotii* alleles at the same loci. This higher allelic diversity in *P. caribaea* than *P. elliotii* suggested there may be less selection for most adventitious rooting loci in *P. caribaea*.

Between-species effects were putatively identified as large effects unique to the F₂

A large effect QTL on group H9 was uniquely detected in the F₂, suggesting this QTL was due to a between-species effect. QTL detected uniquely in the F₂ must be fixed in both parents otherwise segregation would occur in the F₁. Natural selection may be fixing the locus in both parental populations because chance fixation of a locus should be infrequent in outcrossing species and chance fixation of an effect around 10-fold larger than any other QTL should be extremely remote. Large effect QTL (ranging from 20% to 50% PV but as much as 88%) in plants have been previously detected in interspecific F₂ hybrids where parents were divergent for traits involved in adaptation, speciation or domestication (Bradshaw and Stettler 1995; Bradshaw et al. 1995; Paterson et al. 1991; Doebley and Stec 1991).

Where parental populations are subject to divergent selection, the direction of an alleles' effect in derived inbred populations may be predictable if there is no inbreeding depression (Forbes et al. 2004). As *P. elliotii* has a strong capacity to develop adventitious roots, we hypothesised that during adaptation to waterlogged environments, selection may have reduced allelic diversity and fixed some loci in this species. We speculated that *P. caribaea* had not been subject to the same selection and therefore maintained diversity at most loci involved in rooting. However, the detection of a putative between-species effect indicated that some loci in some *P. caribaea* individuals were also fixed. Further, at a between-species effect for adventitious rooting it might be expected that favourable alleles would originate from the *P. elliotii* parent because it was the high performing parent; however, *P. caribaea* provided the favourable allele at the putative between-species effect (large effect QTL on H9). This suggested the locus might be fundamental to root development, involved in the development of both primary as well as secondary

roots. The genetic systems of adventitious and primary rooting overlap extensively (Casson and Lindsey 2003), so the effects of selection on primary rooting may be manifest in the genetics of adventitious rooting.

Inbreeding depression may contribute to genetic variation in the F_2

The identification of QTL in a cross derived from outcrossing parental populations raises the question of whether the detected QTL were a manifestation of inbreeding depression. Conifers, like many outcrossing trees species have high genetic loads and suffer inbreeding depression (Williams and Savolainen 1996; Remington and O'malley 2000). Inbreeding could lead to the detection of "growth" QTL that are merely expression of mutational load carried by parental taxa, rather than "growth" QTL reflective of all inbred and F_2 (Bradshaw and Stettler 1995). The phenotypic variance in populations affected by inbreeding depression may arise from mutations during inbreeding or because of chance fixation of deleterious alleles present in the parental populations (Forbes et al. 2004). Inbred populations of *P. elliottii* × *P. caribaea* typically show signs of inbreeding depression in seedling survival and early field growth. In the current study, the family averages for early field growth and rooting percentages were consistently lower in the F_2 than F_1 families in the same trials, strongly suggesting the influence of inbreeding depression in the F_2 (Shepherd et al. 2005).

Although inbreeding depression was likely to affect the overall vigour and growth in the F_2 , several lines of evidence suggest it was not a major factor contributing to genetic variation attributed to rooting QTL. The strongest evidence for this was that inbreeding depression could not have been a factor contributing to QTL in F_1 population. Deleterious loci linked to QTL could, however, still contribute to variance in the F_2 . A second line of evidence from marker segregation data, suggests that this should not be the case for the QTL of the largest effect on group H9. Markers linked to a recessive lethal show segregation distortion in inbred crosses as these genotypes are purged from the population (Bradshaw and Stettler 1994). On a genetic map of the F_2 parent no significant marker segregation distortion was evident on group H9 where the largest QTL was located, but

distortion was evident at PtTX2126 (group Pe7 P -value <0.005) and PtTX4228 (group H10 P -value <0.05). Inbreeding depression was unlikely to be influencing genetic variances attributable to the QTL on group H9, but may have been a factor at the two other QTL detected in the F_2 .

Implications for breeding and MAS

Our study showed that a greater portion of PV in the F_2 was genetic than in the F_1 , therefore gains from selection should also be higher for this generation. Increases in the genetic variance for the F_2 were due to segregation of large between-species effects. The magnitude of between-species effects suggests it may be more efficient to target one or a few of these effects rather than trying to manipulate many smaller within-species effects. Depending on the economics of the trait and genotyping costs, it may be worthwhile exploiting the strong within-family linkage disequilibrium (LD) created during interspecific hybridisation for MAS, to cull poor rooting genotypes prior to mass propagation of clones and field testing.

One of the limitations anticipated for MAS when applied to single-species tree-breeding population is that the LD expected in outcrossing species will create inconsistent coupling–repulsion phase relationships between marker loci and QTL (Strauss et al. 1992). However, if between-species effects are targeted, MAS may be applicable to population improvement of synthetic hybrids. If loci under strong natural selection can be identified, because selection will reduce allele diversity at a locus and create locus-specific LD (Flint-Garcia et al. 2003), it may be possible to exploit LD to provide relatively consistent coupling–repulsion relationships for MAS in populations of both the parental species (intermated to produce F_1) and synthetic hybrids (intermated to produce F_2 or later generation). Whether useful LD exists at these loci is yet to be determined. In outcrossing plant species such as maize where LD typically declines very rapidly, extensive LD can persist when strong artificial selection is maintained (Palaisa et al. 2003).

Acknowledgements The authors thank R. Mellick, P. Toon and R. Peters for assistance in the nursery, C.G. Williams, D.G. Nikles and P. Bundock for helpful discussions on the manuscript; and the CRC for Sustainable Production Forestry for a Visiting Scientist fellowship for S. Huang.

Appendix 1 *Pinus elliottii* and *P. caribaea* consensus map and listing of markers genotyped in the selective genotyping populations

Marker	Linkage group ^a	Order	Distance cM (K)	F ₁	F ₂
PR048	H1	1	0.0	1	1
PtTX4139	H1	2	14.1	1	1 ^b
PtTX3011	H1	3	16.0		1
SCUPCH48	H1	4	32.5	1	1
PR024	H1	5	33.3		1
APC09	H1	6	36.0		
PtTX3089	H1	7	61.6	1	1
PtTX4112	H1	8	64.3		
PtTX4056	H1	9	64.4		
PR062	H1	10	85.6	1	1
PtTX3091	H2	1	0.0		
PtTX4205	H2	2	21.4	1	1
RPTest20	H2	3	42.7	1	1
NZPR01	H2	4	47.2	1	2
PR118	H2	5	49.4		
PR162	H2	6	54.6	1	1
PR00.46	H2	7	57.8		
PR256	H2	8	82.5	1	1
PR117	H2	9	97.8		
PtTX2006	H2	10	103.6		
PR276	H2	11	105.8		
PtTX4157	H2	12	107.0	1	1
PtTX2037	H3a	1	0.0	1	1
PtTX4004	H3a	2	19.5	1	1
RPTest05	H3a	3	45.5	1	1 ^b
PtTX4033	H3a	4	51.0	1	1
PtTX2094	H3a	5	53.5		
PR031	H3a	6	66.3	1	1
APC15	H3a	7	66.4		
PtTX4046	H3a	8	80.9	1	1
PtTX3106	H3a	9	82.2	1	1
PtTX3103	H3b	10	0	1	1
PtTX3030	H3b	11	5.2		
NZPR07	H3c	12	0.0	1	1
PtTX3127	H3c	13	4.4		
PR043-2	H3c	14	7.9	1	1 ^b
APC03	H3c	15	29.9	1	1
PtTX3020	H4	1	0.0	1	1
PtTX3003	H4	2	5.5		
PtTX4044	H4	3	5.5		
PR005	H4	4	5.5		
PtTX4114	H4	5	15.7		
PtTX3116	H4	6	22.1	1	1
PtTX4009	H5	1	0.0	1	1 ^b
PtTX2080	H5	2	4.6		
PR259	H5	3	11.4	1	1
SPAG7_14	H5	4	15.2		
PtTX4181	H6	1	0.0	1	1
PtTX4030	H6	2	16.1	1	1
NZPR17-1	H6	3	33.5	1	1
PR265	H7	1	0.0	1	1
PtTX4001	H7	2	3.0	1	1 ^c
PtTX4161	H7	3	9.9		

Appendix 1 continued

Marker	Linkage group ^a	Order	Distance cM (K)	F ₁	F ₂
PtTX4071	H7	4	14.3	1	1
PtTX3034	H8	1	0.0	1	1
RPTest11	H8	2	0.0		
PR033-2	H8	3	8.5	1	1
PR111	H9	1	0.0	1	1
PtTX3091	H9	2	4.1		
PtTX3049	H9	3	9.8	1	1
PtTX4058	H9	4	14.0		
PtTX4214	H9	5	19.6	1	1
PtTX4042	H9	6	39.7	1	1
PtTX3107	H9	7	40.3	1	1
PtTX4147	H9	8	69.0	1	1
PtTX3024	H9	9	76.5	1	1
NZPR23	H9	10	90.3	1	1 ^c
PtTX3105	H10	1	0.0	1	1
PtTX4228	H10	2	3.8	1	1
PtTX2082	H10	3	43.4	1	1 ^b
PtTX2146	H10	4	63.3		
PtTX3046	H10	5	84.4		
PtTX3096	H10	6	88.6		
PtTX3120	H10	7	129.7	1	1
RPTest09 ^d	H10	8	142.8		1
PtTX3123	H11	1	0.0	1	1 ^c
APC11	H11	2	23.6	1	1
PtTX3081	H12	1	0.0	1	1
PtTX2123	H12	2	13.7		
NZPR05	H12	3	17.4	1	1
PtTX3013	H13	1	0.0	1	1
PtTX2034	H13	2	2.1		
PtTX4183	H13	3	9.4		
PtTX3025	Pe7	1	0.0	1	1
PtTX4055	Pe7	2	19.2	1	1 ^b
PtTX2126	Pe7	3	21.9	1	1
PtTX4149	Pe7	4	27.8	1	1
PR070	Pe12	1	0.0		
SCUPEE25	Pe12	2	9.8	1	1
PtTX4062	Pc10	1	0.0		
NZPR17	Pc10	2	2.5		
Total	16	91	727.1	56	61
Total polymorphic loci				56	51

^aGrouping and distances (centiMorgan (Kosambi)) were derived from a consensus map of *P. elliottii* and *P. caribaea*. NB. Groups H3 and H10 were fragmented into three groups on species maps. Linkage was inferred from reference to a *P. taeda* map (Zhou et al. 2003). Distances for PtTX3103 (group H3) were uncertain. Order and distances for PtTX2146, PtTX3046 and PtTX3096 (group H10) are from the *P. taeda* map

^bMonomorphic in this family

^cUn-scorable in this family

^dNote: RPTest09 and PtTX2146 are same locus—see Williams et al. (2001)

Appendix 2

Single marker tests were only examined using additive models of gene action. In the F₂ family markers only occurred in an intercross (IC) configuration (using the coding system from Haseman and Elston (1972); see below). The F₁ was more complex, with up to six possible mating configurations (BC1, BC2, MBC, IC, MIC3 and MIC4). Several contrasts could be constructed for some mating types depending on whether contrasts were based on genotypes or alleles. For example in a MIC4 (multiple intercross, 4 alleles segregating) mating configuration each of the four genotypes could be

contrasted. This was referred to as a ‘‘MIC4G’’, i.e. multiple intercross with four alleles segregating, contrasting genotypes. Additionally, for this mating configuration contrasts could be based on allele substitution effects for each parent, ‘‘MIC4E’’, contrasting genotypes pooled on the *P. elliottii* parent and ‘‘MIC4C’’, contrasting genotypes pooled on *P. caribaea* alleles. The contrasts for MIC3, where three alleles segregated, were further complicated by possibility of homoplasmy. We considered models where an allele was identical by state (IBS) or not IBS. Where markers segregated in an IC configuration in the F₁, the only model testable was where alleles were considered IBS.

Contrasts used in Fisher’s exact tests for marker-trait associations in selective genotyping populations derived from an F₁ and F₂. NB—dominance models were not tested in either family

Pedigree	Mating config. ^a	Parental types	Possible progeny contrasts ^b	Contrast code ^c	Tested	
F ₂	IC	A/B×A/B	A/A vs. A/B vs. B/B	IC	Yes	
Outbred F ₁	MIC4	E1/E2×C1/C2	E1/C1 vs. E1/C2 vs. E2/C1 vs. E2/C2	MIC4G	Yes	
			(E1/C1+E1/C2) vs. (E2/C1+E2/C2)	MIC4/C	Yes	
			(E1/C1+E2/C1) vs. (E1/C2+E2/C2)	MIC4/E	Yes	
	MIC3	E1/A×C1/A	Assume A is not IBS then three contrasts as for MIC4		MIC3G	Yes
					MIC3E	Yes
				Assume A is IBS	MIC3C	Yes
				A/A vs. (E1/A+A/C1) vs. E1/C1	MIC3IBS	Yes
	IC	A/B×A/B	Assume A and B are not IBS—untestable Assume either A or B is IBS and the other is not IBS—untestable			No
				Assume A and B are IBS—A/A vs. A/B vs. B/B	ICIBS	Yes
	BC	E1/A×A/A	E1/A vs. A/A		BC	Yes
BC dominant	E1/–×–/–	C1/–×–/–	E1/– vs. –/–	BCdom	Yes	
			C1/– vs. –/–			
MBC	E1/E2×A/A	E1/A vs. E2/A		MBC	Yes	

^aMating configuration codes from Haseman and Elston (1972); BC=backcross; MBC=multiple backcross; IC=intercross, MIC3= multiple intercross with 3 alleles; MIC4=multiple intercross with 4 alleles

Codes E1& E2=alleles 1&2 from *P. elliottii*; C1 and C2=alleles 1&2 from *P. caribaea* and distinguishable by size from E1 and E2; A= is an allele that occurs in both species, likewise for B; –=null allele

^bIBS=Identical by state

^cContrast codes were constructed from the mating type code with the addition of a contrast identifier. G=genotype, where all 4 genotypic means were contrasted; E=elliottii, genotypes were pooled to test the effect of substituting alternative *P. elliottii* alleles; C=caribaea, where the effect of substituting alternative alleles from the *P. caribaea* were contrasted; dom=dominant where marker was treated as a dominant marker

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