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Branch architecture QTL for *Pinus elliottii* var. *elliottii* × *Pinus caribaea* var. *hondurensis* hybrids

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Abstract – Putative quantitative trait loci (QTL) of moderate, additive effect were identified for branch diameter, average number of branches per whorl per tree, average whorl spacing and regularity of whorl spacing in a single *Pinus elliottii* var. *elliottii* × *P. caribaea* var. *hondurensis* family. There was no evidence of additive × additive epistasis or pleiotropy. No marker-trait associations were detected for the average branch angle per whorl per tree. The detection of gene effects that were seemingly larger but less numerous than those expected for traits with low to moderate heritabilities was attributed to bias in the estimation of QTL magnitude and limited power to detect QTL due to a small sample size. Branch architecture traits exhibited considerable variation within the family with ranges of 4–6 standard deviations (SD) and tended to be less variable than height and diameter. Branching characters were largely independent of one another as well as growth, form and wood density properties. Branching characters other than branch angle were not influenced by macro-environmental factors.

genetic mapping / branch angle / branch diameter / whorl spacing / whorl regularity

Résumé – QTL concernant l'architecture de la branchaison chez les hybrides entre *Pinus elliottii* var. *elliottii* × *Pinus caribaea* var. *hondurensis*. On a identifié des QTL qui semblent avoir un effet modéré et additif pour les caractères : (1) diamètre des branches ; nombre moyen de branches par verticille ; espacement moyen entre verticilles et régularité de cet espacement dans une famille de *Pinus elliottii* var. *elliottii* × *Pinus caribaea* var. *hondurensis*. Aucune épistasie additive × additive ou pléiotropie n'a pu être mise en évidence. Il n'a pas été possible non plus de trouver un marqueur associé au caractère angle des branches au niveau verticille et arbre. Les effets des gènes qui ont été détectés peuvent sembler plus importants, et le nombre de gènes moins élevé que ce qu'on pourrait attendre pour des caractères à héritabilité faible à moyenne. Ceci s'explique par la faible dimension de l'échantillon qui induit un biais dans l'estimation de l'effet QTL et limite le pouvoir de détection de ces QTL. La variabilité intra-famille des caractères de branchaison est considérable, avec une amplitude de 4–6 écarts-types ; elle tend à être moins élevée que celle de la hauteur et du diamètre. Ces caractères de la branchaison sont largement indépendants les uns des autres, mais aussi des caractères forme, densité du bois et vigueur. À l'exception de l'angle des branches, ils ne sont pas influencés par les facteurs macro-environnementaux.

cartographie génétique / angle des branches / diamètre des branches / espacement des verticilles / régularité des verticilles

1. INTRODUCTION

Some of the most detailed genetic models for commercial traits in forestry have been provided by mapping their underlying quantitative trait loci (QTL). The need to understand quantitative traits in tree species has driven QTL studies in a range of characters important to forestry, including growth and vigour, wood properties, foliar oil composition,

vegetative propagation traits, branch habit characteristics and physiological traits [1, 5, 9, 13, 14, 26, 32].

Branching architecture in trees is important for wood quantity. It defines the structural basis for photosynthesis surfaces and hence tree productivity [11]. Branching characteristics also influence wood quality as the commercial value of timber may be reduced because knots lower the strength of structural timbers. Knot size is determined by the angle and

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the diameter of the branch [22]. Changing tree form and branching may be the fastest way to improve wood properties because of the higher heritabilities of these characters compared to most wood chemistry characters and their ease of measurement [42]. Knowledge of the genetics underlying tree branching architecture would allow breeders to increase productivity [39].

Exotic *Pinus* spp. plantations comprise around 130 000 ha of the plantation estate in Queensland. Of this, around 100 000 ha may be considered most suited to an interspecific hybrid between *P. elliottii* Engelm var. *elliottii* Little and Dorman (PEE) and *P. caribaea* Morelet var. *hondurensis* Barrett and Golfari (PCH) [16]. The hybrid is the taxon of choice as it combines favourable characteristics from the parents, providing superior growth and form across a range of sites in Queensland [10, 28, 37]. Hybrid pine wood is primarily used for structural timbers, veneer and plywood products [24].

The parents of the hybrid differ markedly in their growth patterns. The PCH parent generally grows fastest as it grows throughout the year, whereas PEE has a distinct period of dormancy followed by a vigorous burst of growth early in the season [35]. As a consequence of their different growth patterns, the two species also have distinctive branching habits [9, 35]. PCH tends to have more regular internode distances and lighter, flatter branches than PEE. PCH also typically has better stem straightness and fewer ramicorns than PEE.

Despite its potential importance to wood properties, little is known about the extent and causes of variation in branch characters in PEE \times PCH hybrids. From the single detailed study carried out to date, on a single second generation hybrid family derived from a self pollination of an interspecific F_1 , compared with height and diameter, branch angle had a similar low coefficient of variation, branch diameter and the number of branches per whorl were moderate, whereas the average number of branches per whorl and regularity of whorl spacing was high [9]. Of those traits that were measured, most indicated continuous, approximately normal distributions except for branch angle, which was tri-modal. This indicates that some branching traits may exhibit relatively high levels of within family variability, that they are largely quantitative but that there may also be large gene effect segregating for some traits in some families.

Evidence from studies of pure species populations of pines indicates that low to moderate amounts of additive genetic control are typical for branching characteristics as narrow sense heritabilities typically range from 0.10 to 0.49, with branch angle amongst the more heritable characters [22, 23, 42]. There is also a general acceptance that the environment is a large factor in determining variability in branching characters and also that in some cases, nonadditive genetic variation can be significant (p. 170 [42]). However, caution is needed in inferring results from pure species to interspecific hybrids. Hybrids offer unique combinations of genes and

issues of incompatibility and differences in the relative importance of dominance and nonallelic interactions arise when comparing hybrids and pure species taxa [5, 27, 38]. Our best indications of the types of genetic control involved in plant architecture may come from QTL studies of interspecific hybrids involving inbred crops. There is strong evidence from a number of independent studies that there are relatively few genes, often of large effect, responsible for the genetic control of traits describing plant and inflorescence architecture in wide hybrids [4, 25]. At present, however, it is unclear whether the genetic control of branch architecture in interspecific pine hybrids will follow a typical quantitative model as with many of the characters of interest to the tree breeder, or, whether the variation will be accounted for by few genes of large magnitude, as has been found for morphological characters defining plant architecture in interspecific crop hybrids.

In this paper we report preliminary identification of QTL for branching architecture in hybrid pines. We sought to understand: (1) the extent and type of genetic control; (2) juvenile-mature correlations; (3) the effect of environment and (4) the relationship of branching traits with other commercial traits. Site effects and phenotypic correlations amongst branching architecture, growth, form, wood density and bark thickness were investigated. Genetic correlations amongst branching architecture traits and with other commercial traits were investigated through co-localization of QTL. We discuss implications for breeding and further experimentation.

2. METHODS

2.1. Population and field experiment

The study is based on a single interspecific F_1 hybrid family from the cross of a PEE seed parent (1EE1-102) and a PCH pollen donor (1CH1-063). The family was planted out within a hybrid progeny trial with a randomised plot design at two sites within south-east Queensland during March 1994. A total of 89 trees, 60 at the Beerburum and 29 at the Tuan site were available for phenotypic assessments. Trees were planted in five-tree line plots with a spacing of 5×2.1 m at Beerburum and 4.5×2.4 m at Tuan. The sites were chosen to represent extremes for the target area for which the hybrid is planted in south-east Queensland. The Tuan site is generally considered less productive, with trees of lower girth and form, due to its lower rainfall (1337 mm versus 1665 mm) and poorer soil (Lateritic podzolic versus deep Red earth) [9, 28]. A further four individuals from a second controlled cross of the same parents were included in the population used to generate a genetic map. These seedlings, however, were too young for phenotypic assessments.

2.2. Phenotypic assessments

Five branch architecture traits were assessed over 3 days in October 2000 similar to those described in Dale 1994.

1. Average branch angle (AVBRA): the mean angle from horizontal of all branches (excluding ramicorns) between breast height (1.3 m) and up to 6 meters but less than 7 whorls from the base of the

tree. The angle was measured using an unclinometer (Suunto) placed at the base of the underside of a branch and reading the angle from horizontal.

2. Average number of branches per whorl (AVBRN): the mean of the number of branches per whorl for whorls defined above.

3. Average branch diameter (AVBRD): the mean diameter at the base of all branches in all whorls defined above. Diameter was measured to the closest millimetre using a micrometer.

4. Average whorl spacing (AVWS): the average distance between major branch whorls as defined above. The height of each whorl was measured with height sticks and the distance between whorls derived by subtraction.

5. Regularity of whorl spacing (CVWS): defined as the coefficient of variation for whorl spacing. This was calculated for each tree as the within tree standard deviation for whorl spacing divided by the mean whorl spacing and expressed as a percentage.

Variables for analysis were derived as an average for each tree over all whorls measured except for AVWS and CVWS.

Three trunk form traits were also assessed.

1. Stem class (SC): assessed subjectively on a scale of 1–6 using the following criteria:

1 = Bend > tree diameter; 2 = bend = tree diameter; 3 = bend = 3/4 tree diameter; 4 = bend = 1/2 tree diameter; 5 = bend = 1/4 tree diameter; 6 = perfectly straight.

2. Ramicorns (RAM): the number of distinctively large, steep angled branches observed per tree.

3. Double leaders (DL): recorded as (0) for trees with a single main leader and (1) for trees with two equal main leaders.

Bark thickness was measured at four positions around the trunk at breast height using a bark gauge (Suunto P/L). An average of the four bark thickness measures (AVBT) was analysed. A further derived variable, relative bark thickness (RBT) was calculated for the proportion of bark in the trunk basal area. Trunk basal area was calculated from diameter at breast height measures:

$$RBT = [(OBDBH)^2 - (UBDBH)^2] / (OBDBH)^2.$$

Growth was assessed at 78 months and 84 months of age at the Beerburum and Tuan sites respectively. Trunk height (HT) was measured using height sticks. Overbark diameter at breast height (1.3 m) (OBDBH) was measured using a circumference tape. Under bark diameter at breast height (UBDBH) was derived by subtracting $2 \times AVBT$ from OBDBH for each tree.

2.3. Descriptive statistics and phenotypic correlations

Traits which were believed to conform to requirements for parametric tests (all traits except DL, RAM and SC were tested for normality (Kolmogorov-Smirnov'KS test) [40]. Site means were compared by t-tests for traits conforming with approximately normal distributions or by a Mann-Whitney test or in a contingency table (DL) for non-normal traits. All tests were carried out using the

Basic Statistics or Nonparametric modules of Statistica v4 (Statsoft, Tulsa, OK).

Site adjusted phenotypic correlations were determined by regressing out site and plot effects and analysing correlations amongst residuals using the multiple regression and basic statistics modules of Statistica. An experiment-wise error level ($\alpha = 0.05$) was applied to correlations using Bonferroni's method [30].

2.4. Genetic maps

A pseudotestcross strategy was used to generate a framework genetic map for each parent based on a sample of 93 F_1 hybrid progeny [15]. Details of genetic map construction are given in Shepherd et al. (in review). Briefly, DNA was prepared from foliage from 93 progeny according to Graham et al. (1994). Amplified fragment length polymorphic (AFLP) markers were generated based on the methodology described in Remington et al. (1999). A total of 299 AFLP markers were identified that segregated in the mapping population in a testcross configuration. Twelve microsatellite markers that transferred from related hard pines and were found to segregate in the mapping population were also included in the linkage analysis [34]. Framework maps were constructed using MapMaker Experimental v3 (MME) and based only on those markers in a testcross configuration [20, 21]. Markers whose parental genotype indicated a testcross mating type yet segregated with a 3:1 ratio in the F_1 progeny were excluded from analysis. Global grouping threshold was set at log of the odds (LOD) 550 cM (K). Best order maps for each linkage group were established first by using the ripple command. A maximum of eight markers per linkage group were rippled simultaneously. Those groups for which the best order was not greater than a LOD 2 relative to the next alternative order were retested after dropping one or more markers. Markers were dropped on the basis of a quality rating and whether they tended to swap order, until an order with an interval support greater than LOD 2 was achieved. The order was then retested by "rippling". With larger groups of greater than eight markers, a subset of high quality, well-spaced markers was chosen from the best order as an initial start group. Other markers were then added to the framework using the "build" command using a threshold LOD linkage of 2. The final order was retested, rippling eight markers or less at a time. The number of markers in each framework map and the map coverage is summarised in *table 1*.

2.5. Composite interval mapping

Marker-trait associations were investigated using composite interval mapping (CIM) with a site co-variate in QTLCartographer v1.14 for the PC [3]. Background markers were selected using the FB option in the Smapqtl module. Experiment-wise error rates were determined empirically using 1000 permutations tests [8]. CIM was carried out using the default criteria in Zmapqtl module of QTLCartographer. QTL are reported where their significance exceeds an experiment-wise error level of 0.05. Two-locus epistatic

Table 1. Genome coverage for framework maps of individual PEE and PCH trees.

Species	No. of groups	No of markers	Total length of groups cM (Kosambi)	Av. spacing ¹	Map length ² cM (Kosambi)	% Genome coverage ³
<i>P. elliotii</i>	23	78	836	15.2	1170	69
<i>P. caribaea</i>	27	109	1283	15.7	1658	95

¹ Average framework marker spacing = sum of length of all linkage groups upon the number of framework marker intervals minus the number of linkage groups [29].

² Map length determined as per Kubisiak et al. (1995) i.e. total length of groups adjusted for 24 true telomeric ends.

³ Based on a genome size of 1700 cM (Kosambi) for *P. taeda* determined by Remington et al. (1999).

interactions (additive \times additive) were investigated amongst markers significantly linked to QTL by testing for significance of interaction effect using a fixed effect model in the ANOVA module of Statistica [5].

2.6. Single-marker tests

A set of 320 markers from either parent which constituted the set of markers used to develop framework maps for the parents were used in single marker tests with RAM, DL and SC. These markers were all mapped in a backcross configuration and although they did not necessarily have 1:1 segregation they did not fit 3:1. 2×2 contingency table tests were used to test for associations with each marker for the traits using the Basic Statistics module of Statistica. An experiment-wise error level 0.05 was applied to each trait using the sequential Bonferroni method which declared the most significant association significant if the pair-wise *P*-value was less than $1.56E-4$.

3. RESULTS

3.1. Frequency distributions and influence of site upon traits

All traits had approximately normal distributions except for SC, RAM and DL (*table II*). RAM, SC and DL variables were not expected to meet requirements for parametric tests and were analysed by non-parametric tests. Traits varied in their ranges, from 4.4 SD for AVBRN to 7.3 SD for HT. Site significantly affected HT, OBDBH, BD, AVBT, SC, RBT and AVBRA. Tuan is regarded as a poor site compared to Beerburum and tends to produce trees with poorer form and growth. The significantly higher averages for HT and DBH traits at Tuan in this study probably were a result of the differ-

ence in age when the two sites were assessed. Hence, in this study the site effect was confounded with an age effect for growth traits. The poorer form at Tuan was evident in the lower SC ratings for this site; however, it was not clear why Tuan should produce trees with thicker bark or more steeply angled branches. Higher average wood density values for the Tuan site were consistent with the expected slower growth rate for this site and a latitudinal effect which causes a higher proportion of latewood (higher density) as pines are grown closer to the equator at the same elevation (Harding, pers. comm.; [41]).

3.2. Phenotypic correlations

Overbark diameter at breast height was highly ($r > 0.7$) positively correlated with UBDBH (*table III*). Tree height was moderately ($0.3 > r > 0.7$) positively correlated with both OBDBH and UBDBH. Average bark thickness was moderately positively correlated with RBT, OBDBH, UBDBH, and AVBRA. Relative bark thickness was moderately negatively correlated with UBDBH. Average whorl spacing was moderately positively correlated with AVBRD. AVBRD was moderately positively correlated with OBDBH and UBDBH.

3.3. Composite interval mapping

A total of 16 putative QTL were detected using CIM with a site co-variate in QTLCartographer (*table IV*). Traits where more than one putative QTL was detected per parent were tested for epistatic interactions. No significant interactions (additive \times additive) effects were detected for any traits (data not shown).

Table II. Descriptive statistics and test for site effects for growth, form, branch architecture and bark thickness traits in a hybrid pine family at two sites, Tuan and Beerburum.

Variable	Overall Mean \pm SD	Range (SD)	KS Test (normal = <i>P</i> -value > 0.05)	Tuan (n = 29) Mean \pm SD	Beerburum (n = 60) Mean \pm SD	Test for site effect	Site effect <i>P</i> -value
AVBT (cm)	1.08 \pm 0.23	4.94	normal	1.27 \pm 0.19	1.00 \pm 0.18	t-test	0.00**
RBT	0.24 \pm 0.04	5.56	normal	0.27 \pm 0.04	0.23 \pm 0.04	t-test	0.00**
SC (1–6)	3.16 \pm 0.92	na	na	2.62 \pm 0.67	3.4 \pm 0.91	Mann-Whitney U	0.00**
RAM (count)	0.68 \pm 0.84	na	na	0.93 \pm 0.96	0.56 \pm 0.75	Mann-Whitney U	0.074
DL (Y/N)	0.08 \pm 0.27	na	na	0.10 \pm 0.31	0.07 \pm 0.25	2×2 contingency table	0.55
AVWS (cm)	66.01 \pm 28.00	5.95	normal	68.89 \pm 33.76	64.60 \pm 24.92	t-test	0.50
CVWS	41.76 \pm 14.097	5.00	normal	43.30 \pm 17.87	41.00 \pm 13.4	t-test	0.50
AVBRA ($^{\circ}$)	35.83 \pm 6.33	4.79	normal	38.03 \pm 5.03	34.75 \pm 6.65	t-test	0.02*
AVBRD (mm)	16.48 \pm 3.14	5.85	normal	16.75 \pm 3.24	16.35 \pm 3.10	t-test	0.58
AVBRN (count)	3.6 \pm 0.68	4.40	normal	3.45 \pm 0.52	3.67 \pm 0.74	t-test	0.16
OBDBH (mm)	16.81 \pm 2.33	6.78	normal	17.63 \pm 2.22	16.40 \pm 2.29	t-test	0.02*
UBDBH (mm)	14.63 \pm 2.12	7.25	normal	15.08 \pm 2.11	14.40 \pm 2.11	t-test	0.16
HT (m)	12.66 \pm 1.27	6.91	normal	13.12 \pm 1.27	12.45 \pm 1.22	t-test	0.02*
BD (kg m $^{-3}$)	368 \pm 26	5.61	normal	384 \pm 24	360 \pm 23	t-test	2E–5**

Table III. Phenotypic correlations adjusted for site and plot for branching, growth, form and bark thickness traits (n = 81). Correlations significant at the experiment-wise level of 0.05 according to Bonferroni's method, are bolded (i.e. pairwise *P*-value < 0.0006) [30]. For trait abbreviations see Methods.

	AVBRN	AVBRA	RBT	SC	DL	RAM	AVWS	CVWS	AVBRD	HT	OBDBH	UBDBH	BD
AVBT	-0.0145	-0.4176	0.6676	-0.0792	-0.0513	-0.1206	0.1572	0.0545	0.2708	0.3350	0.5366	0.3964	0.0326
	<i>P</i> = 0.898	<i>P</i> = 0.000	<i>P</i> = 0.000	<i>P</i> = 0.482	<i>P</i> = 0.649	<i>P</i> = 0.284	<i>P</i> = 0.161	<i>P</i> = 0.629	<i>P</i> = 0.014	<i>P</i> = 0.002	<i>P</i> = 0.000	<i>P</i> = 0.000	<i>P</i> = 0.773
AVBRN		-0.2251	-0.0470	0.2404	0.0314	-0.0742	0.0623	-0.0957	-0.1878	0.0279	0.0404	0.0471	0.0906
		<i>P</i> = 0.043	<i>P</i> = 0.677	<i>P</i> = 0.031	<i>P</i> = 0.781	<i>P</i> = 0.510	<i>P</i> = 0.581	<i>P</i> = 0.396	<i>P</i> = 0.093	<i>P</i> = 0.805	<i>P</i> = 0.720	<i>P</i> = 0.676	<i>P</i> = 0.421
AVBRA			-0.2675	-0.0625	-0.0853	-0.1094	-0.0589	0.0123	0.1296	-0.2448	-0.2579	-0.2016	-0.1831
			<i>P</i> = 0.016	<i>P</i> = 0.580	<i>P</i> = 0.449	<i>P</i> = 0.331	<i>P</i> = 0.601	<i>P</i> = 0.913	<i>P</i> = 0.249	<i>P</i> = 0.028	<i>P</i> = 0.020	<i>P</i> = 0.071	<i>P</i> = 0.102
RBT				-0.0604	0.0171	-0.1799	0.1323	0.0997	-0.0889	-0.1979	-0.2600	-0.4078	0.1216
				<i>P</i> = 0.592	<i>P</i> = 0.879	<i>P</i> = 0.108	<i>P</i> = 0.239	<i>P</i> = 0.376	<i>P</i> = 0.430	<i>P</i> = 0.077	<i>P</i> = 0.019	<i>P</i> = 0.000	<i>P</i> = 0.280
SC					0.1229	-0.1673	0.0494	0.0238	-0.1519	0.0967	-0.0614	-0.0524	0.3026
					<i>P</i> = 0.274	<i>P</i> = 0.135	<i>P</i> = 0.661	<i>P</i> = 0.833	<i>P</i> = 0.176	<i>P</i> = 0.391	<i>P</i> = 0.586	<i>P</i> = 0.642	<i>P</i> = 0.006
DL						-0.0718	-0.1286	0.0080	-0.1996	0.0190	-0.0482	-0.0430	-0.0762
						<i>P</i> = 0.524	<i>P</i> = 0.252	<i>P</i> = 0.944	<i>P</i> = 0.074	<i>P</i> = 0.866	<i>P</i> = 0.669	<i>P</i> = 0.703	<i>P</i> = 0.499
RAM							0.2922	-0.0196	0.1372	-0.0333	0.0709	0.0995	-0.0924
							<i>P</i> = 0.008	<i>P</i> = 0.862	<i>P</i> = 0.222	<i>P</i> = 0.768	<i>P</i> = 0.529	<i>P</i> = 0.377	<i>P</i> = 0.412
AVWS								0.0195	0.5359	0.1753	0.0518	0.0267	-0.0376
								<i>P</i> = 0.863	<i>P</i> = 0.000	<i>P</i> = 0.117	<i>P</i> = 0.646	<i>P</i> = 0.813	<i>P</i> = 0.739
CVWS									0.0728	0.1303	-0.0427	-0.0572	-0.0146
									<i>P</i> = 0.518	<i>P</i> = 0.246	<i>P</i> = 0.705	<i>P</i> = 0.612	<i>P</i> = 0.897
AVBRD										0.1910	0.4430	0.4311	-0.1470
										<i>P</i> = 0.088	<i>P</i> = 0.000	<i>P</i> = 0.000	<i>P</i> = 0.190
HT											0.6529	0.6474	0.0718
											<i>P</i> = 0.000	<i>P</i> = 0.000	<i>P</i> = 0.524
OBDBH												0.9874	-0.0887
												<i>P</i> = 0.000	<i>P</i> = 0.431
UBDBH													-0.1012
													<i>P</i> = 0.368

Ten putative QTL were identified for branching traits. All putative QTL for branching traits had low to moderate (0.19–0.81) standardised gene effects (Δ_1), i.e. the difference in standard deviation units between the two genotypic classes [13]. Two putative QTL for AVWS were identified from the male parent, PCH (*table IV*). The putative QTL were located on separate linkage groups, 14 and 23 and explained 15% and 19% of the genetic variance respectively. The putative QTL were of opposite effect i.e. inheritance of the putative QTL caused the trait to increase at one locus and decrease at the other. The Δ_1 for each putative QTL was 0.54 and 0.60. The difference between the family mean and the genotype with the favorable allele (Δ_2), assuming a wider whorl spacing is favorable, was 0.29 for each locus. The AVWS variable had several outliers, individuals with very high whorl spacing. These outliers contributed to a high S test statistic for these putative QTL due to a poor fit of residuals. To examine whether these outliers disproportionately contributed to the significance of the putative QTL, the outlier values were changed to missing data points and the variable was reanalysed (data not shown). Putative QTL remained

significant without outlier data, hence, the analysis including the outliers is presented.

Three putative QTL were detected for AVBRNO, one from the male and two from the female parent. Two putative QTL for AVBRD were also detected from the female parent. The two putative QTL for branch diameter increased branch diameter when the QTL allele was inherited and combined, the putative QTL contributed to 33% of genotypic variation, assuming additivity of effects. These two putative QTL peaks were located nearby (~8 cM) on the same linkage group and may represent a single QTL as the analysis probably does not allow enough resolution to detect more than one QTL in this region. A single putative QTL for CVWS was identified on the female map linked to the locus a09_154A. This putative QTL allele accounted for 17% of the genotypic variation and tended to reduce variation in whorl spacing.

Putative QTL detected for AVBT, HT, UBDBH, OBDBH and BD were also detected and their effects are summarised in *table IV*. No putative QTL for detected for the variable RBT.

Table IV. QTL detected by composite interval mapping with site co-variate in QTLCartographer. QTL significant at experiment-wise error level of 0.05 determined from 1000 permutations.

Trait ¹	Parent ²	LG ³	Marker ³	Position ⁴	CIL ⁵	CIR	LR ⁶	EW 0.05 ⁷	EW 0.01 ⁷	Add ⁶	R2 ⁶	TR2 ⁶	S ⁶	Het ⁸ (mean)	Het (SD)	Hom ⁸ (mean)	Hom (SD)	Delta 1 ⁹	Delta 2 ⁹
AVBT	M	7	a11_249B	0.01	off end	8.01	14.65	13.92	18.73	0.15	0.11	0.41	3.09	1.02	0.19	1.16	0.24	0.61	0.26
AVBT	M	21	a13_209A	16.01	4.01	26.82	13.96	13.92	18.73	-0.15	0.12	0.43	3.36	1.16	0.22	1.06	0.21	0.43	0.09
AVWS	M	14	a04_243A	38.99	24.22	48.99	14.48	13.00	17.24	28.57	0.15	0.46	133.79	59.07	18.35	74.24	35.13	0.54	0.29
AVWS	M	23	a04r165C	47.37	38.33	53.37	22.72	13.00	17.24	-26.62	0.19	0.43	127.94	74.26	33.54	57.45	17.80	0.60	0.29
AVBRN	M	24	a19_147A	42.01	32.01	53.01	18.65	12.85	16.61	-0.61	0.18	0.28	0.87	3.82	0.64	3.41	0.65	0.60	0.28
HT	M	3	a10_087B	16.01	8.01	off end	29.33	12.92	15.73	1.07	0.21	0.49	0.85	12.48	1.11	13.05	1.06	0.45	0.31
HT	M	17	a24_264A	41.48	37.90	49.40	23.76	12.92	15.73	1.16	0.17	0.50	0.72	12.39	1.10	13.07	1.04	0.54	0.32
HT	M	24	a16_233A	0.01	off end	10.01	20.31	12.92	15.73	0.89	0.13	0.49	0.87	12.47	1.11	12.93	1.09	0.36	0.21
BD	M	20	a20_114A	59.95	52.71	66.87	14.84	13.37	17.26	-19.97	0.14	0.32	7.53	375.00	26.64	360.00	22.66	0.58	0.27
CVWS	F	1	a09_154A	0.01	off end	16.01	19.36	13.20	17.07	12.83	0.17	0.33	5.00	34.89	14.37	47.04	13.52	0.81	0.46
AVBRD	F	16	a02_431A	16.01	0.01	41.91	16.38	12.79	15.72	2.50	0.17	0.29	3.72	15.19	2.29	17.23	3.10	0.65	0.41
AVBRD	F	16	a04_157C	23.67	2.01	33.63	17.19	12.79	15.72	2.43	0.16	0.29	3.34	15.51	2.43	17.50	3.17	0.63	0.31
AVBRN	F	12	a23_092C	22.01	12.01	off end	16.45	13.70	16.53	0.61	0.15	0.36	2.02	3.51	0.63	3.64	0.74	0.19	0.13
AVBRN	F	13	a08_177B	0.01	off end	8.01	14.70	13.70	16.53	0.52	0.12	0.35	1.54	3.47	0.75	3.70	0.62	0.34	0.19
OBDBH	F	14	a03_142B	6.01	off end	20.01	14.20	12.65	15.88	1.69	0.16	0.39	0.06	16.16	2.16	17.79	1.56	0.70	0.42
UBDBH	F	14	a03_142B	4.01	off end	18.01	16.97	12.18	16.83	1.59	0.17	0.38	0.12	14.01	1.96	15.56	1.41	0.73	0.44

¹ Trait abbreviations – see Methods.² Parent – M = male parent *P. caribaea* var. *hondurensis*; F = female parent *P. elliottii* var. *elliottii*.³ LG = linkage group on map. Marker is the name of the marker on the left flanking marker of the QTL peak. Markers are labelled as “a” for AFLP, next two digits are a number which represents an AFLP primer combination followed by a three digit band size (bp). Final letter is a code for band quality; A, B, C = high medium and low respectively. An “r” in the label represents marker linked in reverse phase to the one in which it was scored.⁴ Position = distance in cM (K) from the left telomere.⁵ CIL & CIR = 1 LOD confidence limit on peak position left and right boundaries.⁶ QTL parameters in QTLCartographer; LR = likelihood ratio test statistics; Add = additive genetic effect; R2 = proportion of the variance explained by genetic effects; TR2 = proportion of the total variance explained by the model ie includes covariates; S = test statistic S for normality of the residuals under H₁ [2].⁷ EW 0.05 & EW0.01 are the experiment-wise log likelihood thresholds at the $\alpha = 0.05$ and 0.01 levels determined from 1000 permutations in QTLCartographer [8].⁸ Genotype means for heterozygotes (Het) and homozygotes (Hom) determined from independent t-tests at the marker.⁹ Delta 1 = difference between alternative QTL genotypes expressed in phenotypic standard deviations; Delta 2 = difference between favourable QTL genotype and the population mean expressed in phenotypic standard deviations [13].

3.4. Single Marker Tests

No putative QTL were detected for SC, DL or RAM using single marker tests.

4. DISCUSSION

Exploiting within family variation generated from the crossing of two heterozygous parents, putative QTL influencing branch characteristics, bark thickness, growth and wood density have been mapped in a PEE × PCH hybrid. Branching characters exhibited considerable variation in the family but were typically 1–2 SD less variable than growth traits. Despite lower variances, evidence of genetic control was found in all branching traits except AVBRA. One or two putative QTL of moderate additive effect ($r^2 = 0.1 - 0.2$) were identified for most traits with no evidence of additive × additive epistasis or pleiotropy. Branching characters were generally not influenced by the environment and were largely independent of each other, growth and wood density traits.

Several aspects of the genetic models that are emerging for branching traits required closer evaluation and reconciling

with our expectations of the mode of inheritance and importance of the environment in explaining variability. The design of a QTL detection experiment determines the power to detect QTL as well as the accuracy and precision of the estimates [4]. Small sample sizes can be a major limitation on the power to detect small effect QTL and frequently estimates of their magnitude are overestimated. For example, using simulation analysis on a population of 100 F₂ progeny, Beavis estimated that when there was 10 QTL per trait explaining 63% of the phenotypic variability and no dominance, the power to detect QTL was only 33%. This declined to 9% when 10 QTL accounted for 30% of the phenotypic variation. Furthermore, the estimates of variance explained tended to be inflated, av. 16.76% ± 0.40 when the true value was av. 3%. Hence the power for detection of small effect QTL in our experiment could be quite low as backcross designs have approximately half the power of F₂ [36]. If these power and accuracy limitations were applicable to our experiment, the model of genetic control would be significantly different, indicating the traits are controlled by substantially more genes than we have detected and of smaller effect.

Despite these limitations for detecting small gene effects, our experimental design should have provided adequate power for detecting large, additive gene effects. Simulations of a single QTL per genome with $h^2 = 0.5$ and no dominance on our male map using QTLCartographer, for example, indicated all QTL were detected by CIM for a 100 replicates where an experiment-wise α of 0.05 was applied (data not shown). The power declined to 87% when the h^2 of simulated QTL was reduced to 0.2. Nonetheless, there was no evidence of large gene effects for branching traits either from the QTL study or from multi-modality in frequency distributions. Large gene effects may be more evident in advanced generation hybrids or where there has been inbreeding. Given the lack of evidence for any large effect genes, we conclude that we are probably detecting small effect genes that are overestimated as a consequence of limitations of the experimental design.

A general absence of environmental effects in branching characters is somewhat at odds with previous quantitative studies of the genetics of branching in pines. This may be an indication of the importance of dominance, genotype \times environmental ($G \times E$) factors or other nonallelic interactions in explaining the variation in our hybrid family. Our use of markers segregating in a testcross configuration did not allow us to test for dominance effects in this study, so we could not distinguish between dominance, epistatic (other than $A \times A$) and $G \times E$. In summary, therefore, a working model for the genetic architecture of branching traits in hybrid pines is that these traits are controlled by small effect genes with additive gene action, but that dominance or nonallelic interactions may be important in the expression of some traits.

Branching architecture traits were largely uncorrelated amongst themselves and with other traits, which suggested they would not be greatly affected by selection for growth, form or wood density. A drawback, however, to the lack of inter-correlation amongst branching traits is that selection would need to occur simultaneously on a range of traits. An exception to this lack of correlation was AVBRD and OBDBH. Branch diameter has been found to be positively, hence, adversely correlated with tree vigour (girth and/or height) in at least three studies in pines [6, 23, 35]. This may mean that in selecting for trees on the basis of vigour there will also indirectly select for heavier branching. The lack of co-localization of QTL for these traits, however, suggests that different genome regions influence these traits and that there is potential to break this correlation.

Phenotypic correlation from the current study also indicated that AVBRD had a moderate positive correlation with AVWS. This correlation may result from resource allocation in the tree. A tree laying down thicker, and presumably longer and more productive branches may not need as many whorls (branch diameter and length are positively correlated in pines [6, 22]) or vice versa. Again, however, as AVBRD and AVWS appear uncorrelated at the genetic level, selection for lighter branching may occur independent of selection for whorl spacing.

Failure to identify any QTL for AVBRA was surprising given it tends to be amongst the more heritable (0.3–0.4) branching traits in pines [22, 23]. Possible explanations include; a lack of segregation for genes controlling this trait in this family, incomplete map coverage, low power to detect QTL or differences in the way the trait was assessed compared with other studies. Most studies only measure branch angle on one or two whorls per tree whereas we measured 6 whorls typically. Branch angle tends to vary along the trunk and between major and minor whorls in pines [12]. An equilibrium zone, where branch angle is stabilised, has been recognised in pines and other trees and it has been suggested to characterise the branch angle of a tree, and that whorls should be sampled in this region (references within [12]). It may be important to account for patterns of within tree variation to identify QTL for branch angle and reanalysis of selective branch angle data may be valuable.

The largest branching architecture QTL in the study was detected for CVWS. Regularity of whorl spacing may represent the best prospect for tree improvement of branching characteristics in this family. Regularity of whorl spacing is a consequence of the growth patterns of a tree. PEE has a defined dormancy period and lays down a heavy whorl during vigorous growth at the beginning of the season [35]. This is followed by clusters of subsidiary whorls of lighter branches that develop later in the season. The number of whorls is related to the length of the growth season utilised by the tree. Growth season length tends to be variable in slash pine [35]. Caribbean pine on the other hand, has no defined dormant period and grows throughout the year. Consequently, clear differences in branching patterns are evident between the parental species. PEE tends to have clusters of branch whorls and more irregular whorl spacing compared with a more regular spacing in PCH [9]. The objective for a breeding program may be to select for longer lengths of clear wood, hence, trees with larger CVWS may be more valuable.

5. CONCLUSIONS AND FUTURE WORK

The power of our experiment to detect QTL was limited by the small sample size. Low power may lead to undetected QTL and hence genetic models that are over simplified. Where the power of a QTL detection experiment is low, there is a bias toward detection of QTL of large effect and small effects tend to be overestimated [4, 17, 18]. Furthermore, the detection of spurious QTL increases with small populations due to sampling bias [7] and estimates of QTL parameters may be biased by non-simultaneous estimation methods [18]. These factors indicate that until further analysis can be conducted on a large sample, the models for branching characters should only be considered preliminary and a first glimpse of the genetics of branching architecture in hybrid pines.

The small amounts of additive genetic control identified for most branching traits suggests they will be amongst the

more difficult to improve in hybrid pines. The importance of dominance and nonallelic interactions in controlling these traits is yet to be determined but may be significant as the traits are largely unaffected by the environment. Large scale QTL detection experiments will be required to develop accurate and reliable genetic models as gene effects for branching traits appear to be small. Other families will need to be examined to determine if variation in branching traits are inherently less than growth variables, as detected for the family in this study. Prospects for a rationalisation of the number of branching variables are low due to the apparent lack of redundancy amongst branching and other traits. One way to reduce the high costs of the intensive measurements required for branching may be to reduce the number of whorls assessed, however, care is required to retain repeatability of measures due to the high within tree variation in most branching traits [22]. The surprisingly low influence of environment on most branching traits suggests replication over sites can be minimised.

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