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L. C. Emebiri · M. E. Devey
A. C. Matheson · M. U. Slee

# Interval mapping of quantitative trait loci affecting NESTUR, a stem growth efficiency index of radiata pine seedlings

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**Abstract** NESTUR (needle-to-stem unit rate) is a stem growth index of conifer seedlings that measures the efficiency of stemwood production per unit of needle growth, and is related to other seedling traits such as height, stem diameter, stem volume and needle volume. Quantitative trait loci (QTLs) affecting the expression of stem growth efficiency in radiata pine seedlings were investigated using a RAPD linkage map constructed from markers scored on haploid, megagametophytic DNA. Four putative QTLs were detected which accounted for 8.5–36.4% of the population variance. A search for evidence of epistasis, using both complete pairwise and conditional interactions, did not yield any statistically significant result. Over a 3-year period, seedlings with high-NESTUR marker alleles showed a superior growth performance of 17–40% for height, diameter and volume over those with low-NESTUR marker alleles.

**Key words** NESTUR · Stem growth efficiency · RAPD · QTL · Haploid megagametophyte

## Introduction

A major goal in forest tree breeding is to improve the proportion of carbon assimilates allocated to stemwood production. Only a proportion of the increments of photosynthate is employed to produce wood; the rest is used to produce foliage, roots, and reproductive structures. The proportion partitioned to wood is one of the most important determinants of merchantable wood yield, and is amenable to change by management and genetic selection (Cannell 1989; Arne et al. 1995). However, despite the existence of considerble genetic variation for stem growth efficiency (Cannell et al. 1983; St. Clair 1994), selection in routine breeding programs has not been practical probably because dry matter evaluations are cumbersome to undertake in mature trees, and destructive in seedlings. To permit early selection for stem growth efficiency, Matheson et al. (1995) suggested the use of an index that can be measured non-destructively on seedlings. NESTUR (or needle-to-stem unit rate) is derived from a ratio of unit growth in stem volume to needle volume over time. The index is similar to relative growth rate (RGR) but, unlike RGR, it takes into account the influences of both photosynthetic capacity (as determined by needle volume) and carbon allocation on stemwood production.

The steps involved in the determination of stem growth efficiency are too tedious and time-consuming to permit its use in practical selection programs where thousands of individuals need to be assessed in a very short period of time. Moreover, the genetic basis of the character has not been conclusively established. The heritable basis of such traits can be established by identifying individual loci affecting trait variation or quantitative trait loci (QTLs). In a previous study (Emebiri et al. 1997) we detected marker-QTL linkages in a radiata pine (*Pinus radiata*) full-sib cross using bulked segregant analysis (Michelmore et al. 1991) and random amplified polymorphic DNA (RAPD) markers (Welsh and McClelland 1990; Williams et al. 1990) scored on haploid DNA from megagametophytes. The aim of the present study was to confirm the heritable basis of NESTUR through a genome-wide scan for maternal QTLs in a different family of radiata pine. We also sought to compare the growth of individuals with favourable and unfavourable NESTUR-linked markers under field conditions.

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L. C. Emebiri (✉) · M. E. Devey · A. C. Matheson
CSIRO Forestry and Forest Products, P.O. Box E4008, Kingston ACT 2604, Australia

M. U. Slee
Department of Forestry, The Australian National University, Canberra ACT 0200, Australia

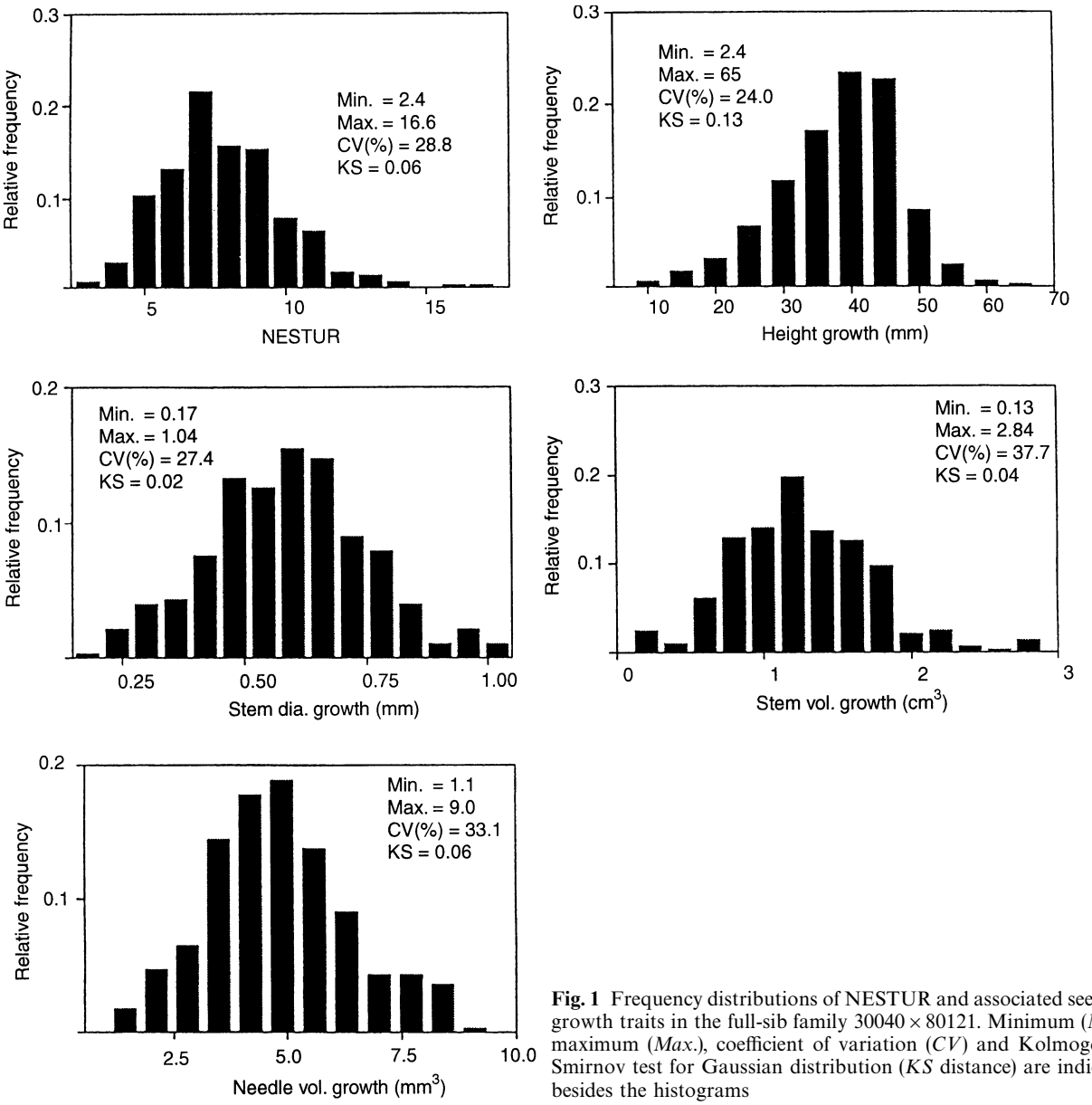


**Fig. 1** Frequency distributions of NESTUR and associated seedling growth traits in the full-sib family 30040 × 80121. Minimum (*Min.*), maximum (*Max.*), coefficient of variation (*CV*) and Kolmogorov-Smirnov test for Gaussian distribution (*KS* distance) are indicated besides the histograms

## Materials and methods

Ninety-three *Pinus radiata* seedlings from a full sib cross (30040 × 80121) were used for this study. Based on previous evaluations, open-pollinated (OP) seeds from the maternal parent (30040) had shown high variance for NESTUR values, while OP seeds from the paternal parent (80121) had a low variance. Germination and megagametophyte rescue were as described in Emebiri et al. (1997).

### Progeny phenotyping

Seedlings were initially grown in a glasshouse under ambient temperature and light conditions for 2 weeks, before transfer to a controlled environment (Phytotron). The growth environment was maintained at 25°C (maximum or day) and 18°C (minimum or night) temperatures, and a relative humidity of 65%. Nutrients were provided in the irrigation water through drippers at 12-h intervals.

From 4 months of age, separate measurements were made on each of two dates for the following traits: (1) total height, measured from the soil level to the growing point, (2) trunk height, from soil level to the cotyledonary scar, (3) stem diameter, gauged with a dendrometer (0.001 mm resolution), and (4) needle volume. Stem volume was calculated as basal area × total height while needle volume, derived using a water displacement method, was calculated as the volume of water displaced minus the volume occupied by stemwood in crown-height. NESTUR values were calculated for each individual using the formula:

$$N = [(SVt_2 - SVt_1)/(NVt_2 - NVt_1)] \times [(\ln NVt_2 - \ln NVt_1)/T_2 - T_1)],$$

where SV = stem volume ($mm^3$), NV = needle volume ($mm^3$), T = time of trait measurement (15 days apart from commencement). Seedling growth in height (HTgr), diameter (SDgr), volume (SVgr) and needles (NVgr) were determined as the difference between two measurements taken at an interval of 2 weeks.

After measurements for NESTUR, seedlings were planted out in the field and monitored for growth performance. Data were

collected over a 3-year period on various traits including: (1) total height, measured from ground level to the tip of the growing shoot, (2) basal diameter, the average of two measurements taken at right angles from 20 mm above ground level, and (3) stem volume, calculated as total height × basal area.

### Map construction and QTL analysis

Total genomic DNA was extracted from megagametophytes according to Dellaporta et al. (1983). The haploid DNAs of the 93 progeny were then genotyped for RAPD markers, using the methods described in Emebiri et al. (1997). The methods employed in linkage map construction are described in a subsequent paper (Emebiri et al. 1998). The final map was constructed with 222 markers, with 4.9% missing genotypes, and covered a total distance of 1665 cM. The maps provided a resolution of one marker every 8 cM, on average (Emebiri et al. 1998).

### Data analysis

QTL analyses were first performed using simple linear regression, where the value of the character for each individual is the regressed variable and the allelic composition of markers is the regressor, assuming a value of 1 for absence and 2 for presence. Comparison-wise error thresholds were calculated from 1000 permutations of the phenotypic data, which provided critical values for each marker but did not account for repeated testing over the genome. Permutations were carried out with the aid of the computer program QTL CARTOGRAPHER, developed by Basten et al. (1997).

Interval mapping was then performed using the approximate multiple-QTL (composite) method of Zeng (1994) and Jansen and Stam (1994), as described in Emebiri et al. (1998). To account for repeated testing over the entire genome, error threshold values were estimated from 1000 permutations of the data for each trait.

### Effects of NESTUR-linked QTLs on field growth performance

To assess the effects of nominated QTLs on field growth performance, the mapping population was divided into two genotypic classes, based on their allelic configuration for markers closest to putative QTLs identified for NESTUR. One genotypic class comprised individuals positive for favourable marker alleles, and the other group negative. A two-way ANOVA was used for data analyses to account for year-to-year variation in growth, according to the following statistical model: $Y_{ijk} = m + G_i + Y_j + GY_{ij} + E_{ijk}$, where $Y_{ijk}$ is the observed phenotype, m is the overall mean, $G_i$ and $Y_j$ are the main effects of the genotypic class and year, respectively; $GY_{ij}$ is the interaction term and $E_{ijk}$ is the residual effect.

## Results

### Frequency of phenotypes

NESTUR had a mean value of 7.8, with a minimum of 2.5 and a maximum of 16.6. There was substantial phenotypic variation among the $F_1$ progenies for all traits. The phenotypic coefficient of variation (CV) for NESTUR was 28.8%. For stem growth traits, the CV ranged from 24% for height growth (HTgr), to 37.7% for stem volume growth (SVgr). The five traits showed continuous variation (Fig. 1), exhibiting a nearly

**Table 1** Linkage group and RAPD marker loci that showed significant association with NESTUR and stem growth traits from simple linear regression analyses. Only markers with an *F*-probability at or above the comparison-wise threshold are reported

| Linkage group | Marker locs | NESTUR | | | SDgr | | | SVgr | | | HTgr | | | NVgr | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *F*-prob | Allelic effect[a] | $R^2$ (%) | *F*-prob | Allelic effect | $R^2$ (%) | *F*-prob | Allelic effect | $R^2$ (%) | *F*-prob | Allelic effect | $R^2$ (%) | *F*-prob | Allelic effect | $R^2$ (%) |
| 1 | OPE09_1000 | 0.002 | − 0.63 | 12.4 | – | – | – | – | – | – | – | – | – | – | – | – |
| 3 | OPAB18_450 | – | – | – | 0.001 | 0.61 | 11.1 | – | – | – | – | – | – | – | – | – |
| 3 | BC316_300 | – | – | – | 0.001 | 0.66 | 12.6 | 0.003 | − 0.58 | 7.7 | – | – | – | – | – | – |
| 3 | OPX04_600 | – | – | – | – | – | – | – | – | – | – | – | – | 0.002 | 0.59 | 9.5 |
| 4 | OPA08_425 | 0.001 | − 0.70 | 13.5 | – | – | – | – | – | – | – | – | – | – | – | – |
| 4 | OPV17_350 | < 0.001 | 0.73 | 15.5 | < 0.000 | 0.71 | 13.9 | < 0.000 | 0.74 | 15.4 | 0.001 | 0.59 | 9.4 | – | – | – |
| 6 | OPB11_950 | – | – | – | 0.000 | − 0.58 | 9.3 | 0.003 | − 0.57 | 7.3 | – | – | – | 0.001 | − 0.7 | 13.3 |
| 12 | OPZ09_800 | 0.003 | 0.57 | 8.6 | – | – | – | – | – | – | 0.001 | 0.60 | 10.1 | – | – | – |
| 12 | BC533_1100 | – | – | – | – | – | – | – | – | – | 0.001 | 0.65 | 11.8 | – | – | – |

[a] Allelic effect is the difference between the two QTL allele phenotypes in standard deviation units

normal distribution (as measured by the Kolmogorov-Smirnov test) without transformation.

## QTL detection

Results of the simple linear regression analyses (Table 1) showed good agreement with those obtained from interval mapping (Table 2). Both methods detected four putative QTLs for NESTUR in linkage groups 1, 4 and 12. The putative QTL in group 1 was closest to marker OPE09_1000, and explained 9.6% of the observed variation, while that on group 12 (closest to marker OPZ09_800) explained 8.5% of the variation. Two peaks were observed in group 4 where simple linear regression had indicated two clusters of significant marker loci associated with NESTUR. Based on the opposing direction of their associated effects, there was evidence of two putative QTLs for NESTUR in this region. One of the QTLs, that closest to marker OPA08_425, had a large effect on NESTUR, explaining 36.4% of the population variance. The four putative QTLs cumulatively explained 71% of the observed variation in NESTUR, but this value is probably upwardly biased.

## Genetic relationships between NESTUR and stem growth traits

Because NESTUR was not actually measured but derived from other measured traits, we sought to determine whether inferred QTLs were identical to those affecting the component traits. A summary of descriptive data for all putative QTLs affecting stem growth traits are presented in Tables 1 and 2, alongside those for NESTUR. NESTUR showed a highly significant correlation with all measured traits (Table 3). Much of the positive correlations with SDgr and SVgr can be attributed to the influence of the two putative QTLs detected for NESTUR in group 4, particularly the QTL position closest to marker OPV17_350 (Fig. 2; Tables 1 and 2). The putative QTL with a large effect on NESTUR (that closest to OPA08_425) also had a large effect on stem diameter growth (SDgr), explaining 22% of the observed variation (Table 2). In linkage group 12, the LOD scores for height increment (HTgr) were maximal in a region adjacent to that harbouring a putative QTL for NESTUR (Fig. 2). Thus, while some putative QTL positions for NESTUR appeared to be identical to those for its components, there were some significant QTLs not shared with the component traits.

A similar pattern of coincidence was found for the other "derived" traits (Table 2). For needle volume increment (NVgr), for instance, we detected three putative QTLs in linkage groups 3 and 6. All putative QTLs were detected in genomic regions with significant effects on SDgr, SVgr and HTgr (Tables 1 and 2). However, there was insufficient evidence to suggest that either of the two linkage groups harboured a putative QTL for NESTUR.

A search for evidence of epistasis, both complete pair-wise and conditional interactions (Chase et al. 1997), did not yield any statistically significant result, suggesting that epistasis may not be a major factor contributing to the observed phenotypic variation for these traits in this cross.

## Effect of NESTUR-linked markers on seedling growth

We compared the 3-year growth performance of seedlings with high- and low-NESTUR marker alleles, using a two-way ANOVA model to account for the effect

**Table 2** Summary of location and properties of putative QTLs affecting NESTUR and seedling growth traits in radiata pine family 30040 × 80121 as determined by approximate multiple-QTL mapping

| Linkage group | Closest marker | NESTUR | | SDgr | | SVgr | | HTgr | | NVgr | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Peak LOD | Var (%) | Peak LOD | Var (%) | Peak LOD | Var (%) | Peak LOD | Var (%) | Peak LOD | Var (%) |
| 1 | OPE09_1000 | 3.55 | 9.6 | – | – | – | – | – | – | – | – |
| 2 | BC333_550 | – | – | 2.52 | 5.5 | – | – | – | – | – | – |
| 2 | BC533_1150 | – | – | – | – | 3.03 | 7.4 | – | – | – | – |
| 3 | OPAB18_450 | – | – | 3.98 | 10.3 | 3.87 | 9.7 | – | – | – | – |
| 3 | OPX04_600 | – | – | 3.71 | 10.0 | 4.60 | 11.6 | – | – | – | – |
| 3 | BC668_650 | – | – | – | – | – | – | – | – | 5.19 | 11.5 |
| 4 | OPV17_350 | 3.61 | 16.4 | 3.08 | 8.8 | 3.98 | 8.4 | – | – | – | – |
| 4 | OPA08_425 | 5.42 | 36.4 | 3.85 | 22.2 | – | – | – | – | – | – |
| 4 | BC366_1300 | – | – | – | – | – | – | 2.90 | 7.1 | – | – |
| 6 | OPAM12_450 | – | – | – | – | – | – | – | – | 2.77 | 5.9 |
| 6 | OPB11_950 | – | – | – | – | – | – | – | – | 4.60 | 10.8 |
| 6 | BC295_800 | – | – | – | – | – | – | 3.97 | 9.2 | – | – |
| 12 | OPZ09_800 | 3.56 | 8.5 | – | – | – | – | – | – | – | – |
| 12 | BC533_1100 | – | – | – | – | – | – | 3.55 | 12.1 | – | – |

**Table 3** Spearman rank correlation coefficients for NESTUR and associated seedling growth traits

| Item | NESTUR | SDgr | SVgr | NVgr |
|---|---|---|---|---|
| Diameter increment (SDgr) | 0.76** | – | | |
| Stem vol. increment (SVgr) | 0.75** | 0.89** | – | |
| Needle vol. increment (NVgr) | 0.26* | 0.55** | 0.70** | – |
| Height increment (HTgr) | 0.61** | 0.56** | 0.74** | 0.58** |

of years. The results, presented in Table 4, showed that the two classes of individuals were significantly different ($P \leqslant 0.05$) in height, stem diameter, stem volume and internode length attained at each of the 3 years of measurement. Interactions of the genotypic classes with year-to-year variation were not significant for any of the measured traits, indicating consistency in the superior performance of seedlings with favourable NESTUR-linked marker alleles. On average, seedlings with high-NESTUR marker alleles showed a superior growth performance of 17–40% over those with low-NESTUR marker alleles. Differences in the number of whorls, branches and crown-height (i.e. the distance from the first whorl to the tip of the growing shoot) were not significant, suggesting that while the nominated QTLs for NESTUR had no effect on the capacity for carbon assimilation, they did influence the efficient distribution of assimilated carbon to stemwood production.

## Discussion

The detection of quantitative trait loci affecting stem growth efficiency is consistent with the carbon allocation pattern of trees determined by physiologists and quantitative geneticists. Past studies have shown that stemwood formation results from cambial activities that are mediated by leaf development (Kozlowski 1971). The greater the number of leaves, the greater the wood production. However, stem increment per unit amount of foliage decreases with an increasing amount of foliage per tree. This is largely because the amount of branchwood produced by a unit amount of foliage increases with an increasing amount of foliage per tree. If respiratory losses by non-green tissues were held constant, differences in the foliage efficiency of trees in producing wood would largely be caused by differences in the distribution of dry matter in various parts of the trees.

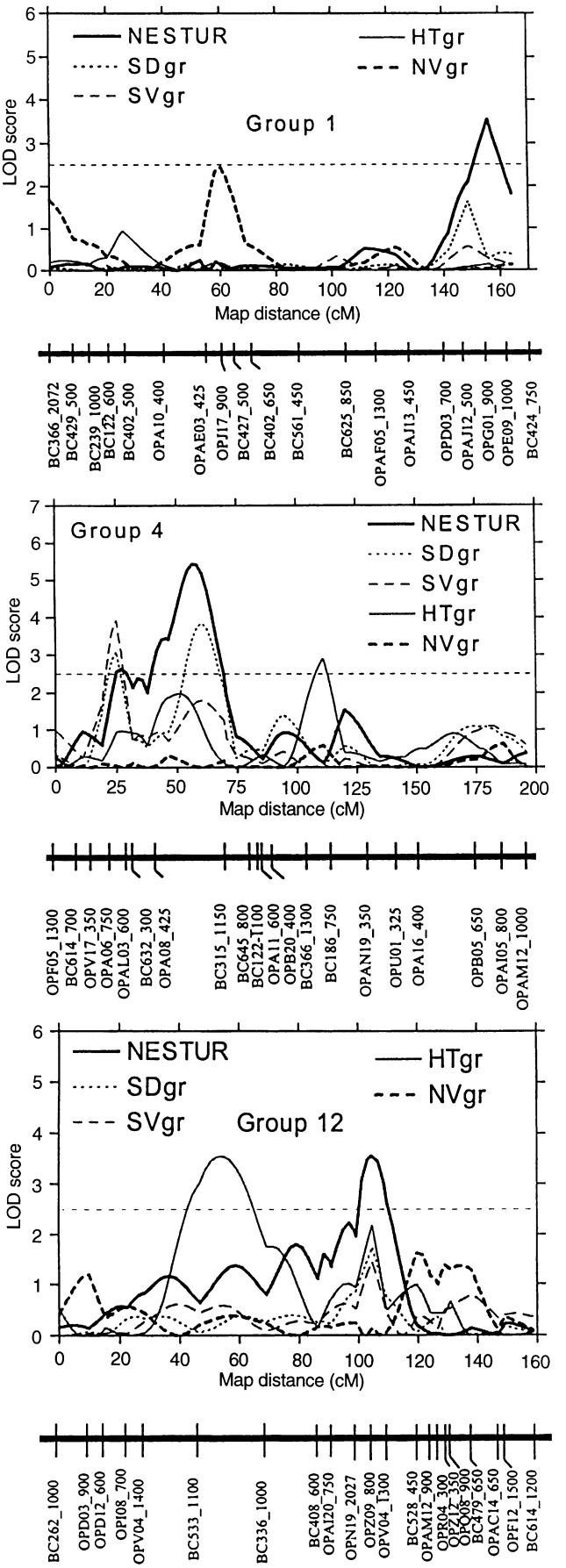


**Fig. 2** Likelihood profiles obtained for NESTUR and stem growth traits in linkage groups 1, 4 and 12. The LOD scores were calculated using an approximate multiple-QTL model with the aid of MAPQTL. The dotted horizontal lines indicate the 5% significance threshold

**Table 4** ANOVA of marker genotpye class and year effects on radiata pine seedling growth performance under field conditions

| Source of variation | *df* | Stem diameter (mm) | Stem volume ($m^3$) | Height (m) | Crown-height (m) | Internode length (m) | Number of whorls | Number of branches |
|---|---|---|---|---|---|---|---|---|
| Genotypic class (G) | 1 | 672.05** | 7.01* | 1.20** | 0.16ns | 0.04** | 2.61ns | 81.71ns |
| Years (Y) | 2 | 10249.34** | 47.76** | 12.95** | 13.85** | 0.42** | 83.72** | 2893.22** |
| G × Y | 2 | 12.08ns | 0.55ns | 0.01ns | 0.02ns | 0.01ns | 0.84ns | 33.22ns |
| Residual | 49 | 93.20 | 1.38 | 0.15 | 0.11 | 0.004 | 1.11 | 66.73 |
| Means[a] | | | | | | | | |
| Marker (+) | | 48.1 | 2.53 | 1.88 | 1.16 | 0.26 | 4.7 | 34.3 |
| Marker (−) | | 41.0 | 1.81 | 1.59 | 1.05 | 0.20 | 5.1 | 31.8 |
| $LSD_{0.05}$ | | 5.2 | 0.63 | 0.21 | 0.18 | 0.03 | 0.6 | 4.4 |
| % Diff. | | 17.3 | 39.80 | 18.20 | 10.50 | 30.00 | − 7.8 | 7.9 |

**, * Indicates effects that were statistically significant at 1% and 5% levels of error probability; ns indicates effects that were not statistically significant

[a] Marker (+) indicates mean of seedlings positive for high-NESTUR alleles; Marker (−) indicates mean of seedlings with low-NESTUR marker alleles

Earlier genetic studies on the growth efficiency of trees relied on biometrical methods (Cannell et al. 1983; Velling and Tigerstedt 1984; St. Clair 1994). The use of molecular markers now provides an alternative method to study this variation and to test the assumption that variation may involve many genes, each with a relatively small contribution to the phenotype (Bradshaw 1996). Our results argue for the existence of a genetic system with a relatively small number of loci that cause most of the phenotypic variation. Although some of the detected putative QTLs had effects of $\leqslant 10\%$, it is important to note that these are only maternal QTLs. The power to detect QTLs in the present study was probably low due to our use of dominant markers segregating on only one side of the pedigree. Dominance of markers will lead to lower LOD scores (Ooijen 1992) and also to an under-estimation of the magnitude of explained variation if multiple alleles are segregating at a locus in offspring of out-breeding species (van Eck et al. 1994). With co-dominant markers, there is always the possibility that paternally segregating alleles could enhance the effects of maternal alleles through intra-locus interactions, as demonstrated by Groover et al. (1994) and Byrne et al. (1997).

There are now several reported studies on QTL analysis of quantitative traits in forest trees, but a key issue of the need to distinguish true positive results from false positives remains. Repeating experiments using independent samples from the same or a different cross can assess the validity of nominated QTLs. In the present study, we have used a different radiata pine cross to repeat an analysis of marker-QTL linkage for NESTUR reported in a previous study (Emebiri et al. 1997). Of the three RAPD markers reported in Emebiri et al. (1997), OPA10_1200 was monomorphic in the present population, while OPE06_450 appeared to be segregating but was difficult to visualise, even after Southern blotting. Only the marker UBC333_550 could be scored unambiguously, and was mapped to linkage group 2 in a region harbouring a putative locus for SDgr (Table 2).

This result adds to the general agreement among scientists that RAPD markers may not be easily scored across populations. However, the detection of QTLs affecting NESTUR in both studies confirms the genetic basis of the trait. In the present study, we also compared the field growth performance of seedlings with favourable and unfavourable NESTUR-linked marker alleles to assess correlated effects of nominated QTLs. Because NESTUR can only be measured most conveniently at an early stage of growth (4–6 months of age), the effects of the nominated QTLs were assessed using growth performance for height, stem diameter and stem volume. If the detected markers were indeed linked to QTLs influencing stem growth efficiency, then individuals positive for high-NESTUR marker alleles would be expected to show a superior performance in height, stem diameter and stem volume. A comparison of these individuals with those homozygous for low-NESTUR marker alleles (Table 4) provided further confirmation for the genetic basis of the trait for stem growth efficiency improvement in radiata pine.

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## References

Arne A, Lennart B, Ola R, Erik S (1995) Traits related to stem volume yield-capacity in sample trees of *Pinus sylvestris* L. and *Pinus contorta* Dougl. Scand J For Res 10:120–128

Basten CJ, Weir BS, Zeng Z-B (1997) QTL CARTOGRAPHER, a reference manual and tutorial for QTL mapping. Department of Statistics, North Carolina State University, Raleigh, North Carolina, USA

Bradshaw HD Jr (1996) Molecular genetic of *Populus*. In Biology of *Populus* and its implication for management and conservation. Part 1, Chapter 8. In: Stettler RF, Bradshaw HD Jr, Heilman PE, Hinckley TM (eds), NRC Res Press, pp 183–199

Byrne M, Murrell JC, Owen JV, Kriedeman P, Williams ER, Moran GF (1997) Identification and mode of action of quantitative trait loci affecting seedling height and leaf area in *Eucalyptus nitens*. Theor Appl Genet 94 : 674–681

Cannell MGR, Sheppard LJ, Ford ED (1983) Clonal differences in dry matter distribution, wood specific gravity and foliage efficiency in *Picea sitchensis* and *Pinus contorta*. Silvae Genet 32 : 5–6

Cannell MGR (1989) Physiological basis of wood production: a review. Scand J For Res 4 : 459–490

Chase K, Adler FR, Lark KG (1997) Epistat: a computer program for identifying and testing interactions between pairs of quantitative trait loci. Theor Appl Genet 94 : 724–730

Dellaporta SL, Wood J, Hicks JB (1983) A plant DNA minipreprraration: version II. Plant Mol Biol Rep 1 : 19–22

Eck HJ van, Jacobs JME, Stam P, Ton J, Stiekema WJ, Jacobsen E (1994) Multiple alleles for tuber shape in diploid potato detected by quantitative genetic analysis using RFLPs. Genetics 137 : 303–309

Emebiri LC, Devey ME, Matheson AC, Slee M (1997) Linkage of RAPD markers to NESTUR, a stem growth index of radiata pine seedlings. Theor Appl Genet 95 : 119–124

Emebiri LC, Devey ME, Matheson AC, Slee M (1998) Age related changes in the expression of QTL for growth in radiata pine seedlings. Theor Appl Genet (in press)

Groover A, Devey M, Fiddler T, Lee J, Megraw R, Mitchel-olds T, Sherman B, Vujcic S, Williams C, Neale D (1994) Identification of quantitative trait loci influencing wood specific gravity in an outbred pedigree of loblolly pine. Genetics 138 : 1293–1300

Jansen RC, Stam P (1994) High resolution of quantitative traits into multiple loci via interval mapping. Genetics 136 : 1447–1455

Kozlowski TT (1971) Growth and development of trees. Academic Press, New York

Matheson AC, Spencer DJ, Kriedmann PE (1995) Age-age correlation and early selection in radiata pine. 1. Family × environment interactions in plantation and greenhouse. Aust For 58 : 35–43

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions using segregating populations. Proc Natl Acad Sci USA 88 : 9828–9832

St. Clair JB (1994) Genetic variation in tree structure and its relation to size in Douglas-Fir. 1. Biomass partitioning, foliage efficiency, stem form and wood density. Can J For Res 24 : 1226–1235

Velling P, Tigerstedt PMA (1984) Harvest index in a progeny test of Scots pine with reference to the model of selection. Silva Fennica 18 : 21–32

Welsh J, McClelland M (1990) Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res 18 : 7213–7218

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18 : 6531–6535

Zeng Z-B (1994) Precision mapping of quantitative trait loci. Genetics 136 : 1457–1468