Theor Appl Genet (1998) 97: 1053–1061 

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

# Age-related changes in the expression of QTLs for growth in radiata pine seedlings

Received: 10 July 1997 / Accepted: 31 March 1998

**Abstract** Haploid megagametophytes from a full-sib cross of *Pinus radiata* were used to construct a genetic linkage map for radiata pine based on random amplified polymorphic DNA (RAPD) markers. The map, which was made from 222 markers, was used to carry out a QTL analysis of growth using measurements made on seedlings from which these megagametophytes were collected at an early germination stage. Trends in the expression of QTLs for stem diameter, volume and height were compared using measurements made at 5 months of age, and at 1, 2 and 3 years of age. None of the observed trends showed complete stability, i.e. none of the putative QTL positions detected at any one age was strongly expressed at all of the four stages of measurement. However, 45% of the trends showed partial stability, i.e. putative QTLs significant at one age were also detected at a subsequent age. Trends in QTL expression with age followed one of three patterns: (1) putative QTLs at some locations showed a gradual linear increase in influence from 5 months of age and were highest at 3 years of age; (2) QTLs detected at 5 months of age gradually became less significant with age; and (3) some putative QTLs showed a curvilinear increase in effect from 5 months of age, reaching their peak expression at 1 to 2 years, and sometimes were still detected at 3 years of age.

**Key words** RAPD · QTL · Age effects · Stem growth · Linkage map · Haploid megagametophyte

Communicated by G. E. Hart

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

## Introduction

A major problem in forest-tree breeding is the changes in the genetic control of growth that occur during the long life cycle of trees. Growth assessment and selection at a stage well before rotation would markedly increase the efficiency of tree improvement. However, age-related changes make it difficult to predict mature characteristics from juvenile traits and, so far, efforts at developing early testing criteria have met with limited success (see reviews by Greenwood and Volkaert 1992).

Marker-assisted selection (MAS) methods can improve the accuracy of early selection by direct identification of individuals carrying quantitative trait loci (QTLs) for superior later-age growth. Recent reports on the genetic architecture of superior growth, i.e. the number, chromosomal location, mode of action and magnitude of effects of QTLs, increase enthusiasm about the potential of MAS as a breeding tool (Bradshaw and Stettler 1995; Grattapaglia et al. 1996; Byrne et al. 1997). However, QTL expression may not be stable across years of growth. The genetic control of growth may vary with time, such that progenies showing good growth in the initial stages may not necessarily be performing the same in subsequent years.

Past studies on the molecular genetics of stand development in forest trees have concentrated on the most obvious example of change: the shift from vegetative to reproductive development (see reviews by Hutchinson and Greenwood 1991). However, many significant changes also occur in stem growth as the seedling progresses from the embryonic to the post-embryonic (i.e. the vegetative) stage of development. Plomion et al. (1996) recently reported the variable expression of QTLs for height in maritime pine grown for 2 years under accelerated growth conditions.

In this study, we have constructed a linkage map of random amplified polymorphic markers (RAPDs) (Welsh and McClelland 1990; Williams et al. 1990)

scored on haploid DNA of radiata pine (*Pinus radiata*). By co-segregation of these markers with growth traits measured over a 3-year period, we sought to determine whether QTLs at similar chromosomal locations accounted for observed variation in the growth of seedlings under field conditions.

## Materials and methods

### Plant materials

*P. radiata* seeds from the full sib cross 30040 × 80121 were obtained from CSIRO Forestry and Forest Products. To ensure uniform germination, approximately 300 seeds of the $F_1$ full-sibs were placed in a Petri dish lined with three layers of filter paper and stratified for 30 days at 4°C. Ninetythree of the germinants were picked out onto 150-mm-diameter pots filled with a porous medium and grown for 10 days before the megagametophytes were rescued and stored at − 80°C for subsequent DNA extraction.

### DNA extraction and RAPD screening

Total DNA was extracted from megagametophytes according to Dellaporta et al. (1983). To identify primers which amplified polymorphic bands, DNA from six megagametophytes were tested with an array of 432 primers, consisting of 194 Operon primers supplied by Operon Technologies, Alameda, Calif., and 238 primers from the "conifers RAPD set" obtained from Dr. John Carlson, University of British Columbia. Based on the number of polymorphic bands produced, and on their sharpness, 132 primers were selected for genetic linkage-map construction. All of the chosen primers were used to amplify DNA of the mapping population, which consisted of 93 progenies from the cross, including the six already tested and used this time as a repeatability assay.

### Linkage-map construction

RAPD reaction mixtures and assay conditions were as described in Emebiri et al. (1997). A total of 267 polymorphic markers that satisfied a 1 : 1 segregation test ($P \leq 0.05$) were initially employed for linkage-map construction as a backcross dataset, using JOINMAP ver. 2.0 (Stam and Ooijen 1995). Linkage groups were formed with a LOD of 4.0, while the linear order within each linkage group was determined with a 'jump' of 3 and a triplet value of 8. A ripple was performed after the addition of every three markers, and map distances calculated using the Kosambi function.

### Progeny phenotyping

Seedling trees were planted out in the field after 5 months of growth in the glasshouse. On each individual seedling tree, trait measurements were made at 5 months, and then at 1, 2 and 3 years of age. Total height was measured from ground level to the tip of the growing shoot. Basal diameter was measured as the average of two measurements taken at right angles from 20 mm above ground level. Stem volume was estimated as total height × stem basal area.

### QTL mapping

The chromosomal locations of putative QTLs were determined by the method of interval mapping (Lander and Botstein 1989) using the approximate multiple-QTL (composite) method of (Jansen and Stam (1994) and Zeng (1994)). The approximate multiple-QTL method is a two-stage procedure in which, in the first stage, important markers are selected in a step-wise multiple regression of the phenotype on all markers. The second stage involved interval mapping with the selected markers used as co-factors to absorb the major part of variation induced by QTLs located elsewhere in the genome.

The analyses were performed with the MAPQTL program of Ooijen and Maliepaard (1996) using 15 pre-selected markers. Evidence for a single putative QTL was assessed every 2 cM on the genome, and when the LOD score exceeded a genome-wide threshold, the evidence was considered significant. The genome-wide critical threshold for $\alpha = 0.05$ was estimated with the aid of QTL CARTOGRAPHER (Basten et al. 1997) from 1000 permutations of the phenotypic data for each trait. The error threshold obtained from permuted data for each trait varied only slightly, and a mean value of 2.5 was chosen to provide a common significance threshold.

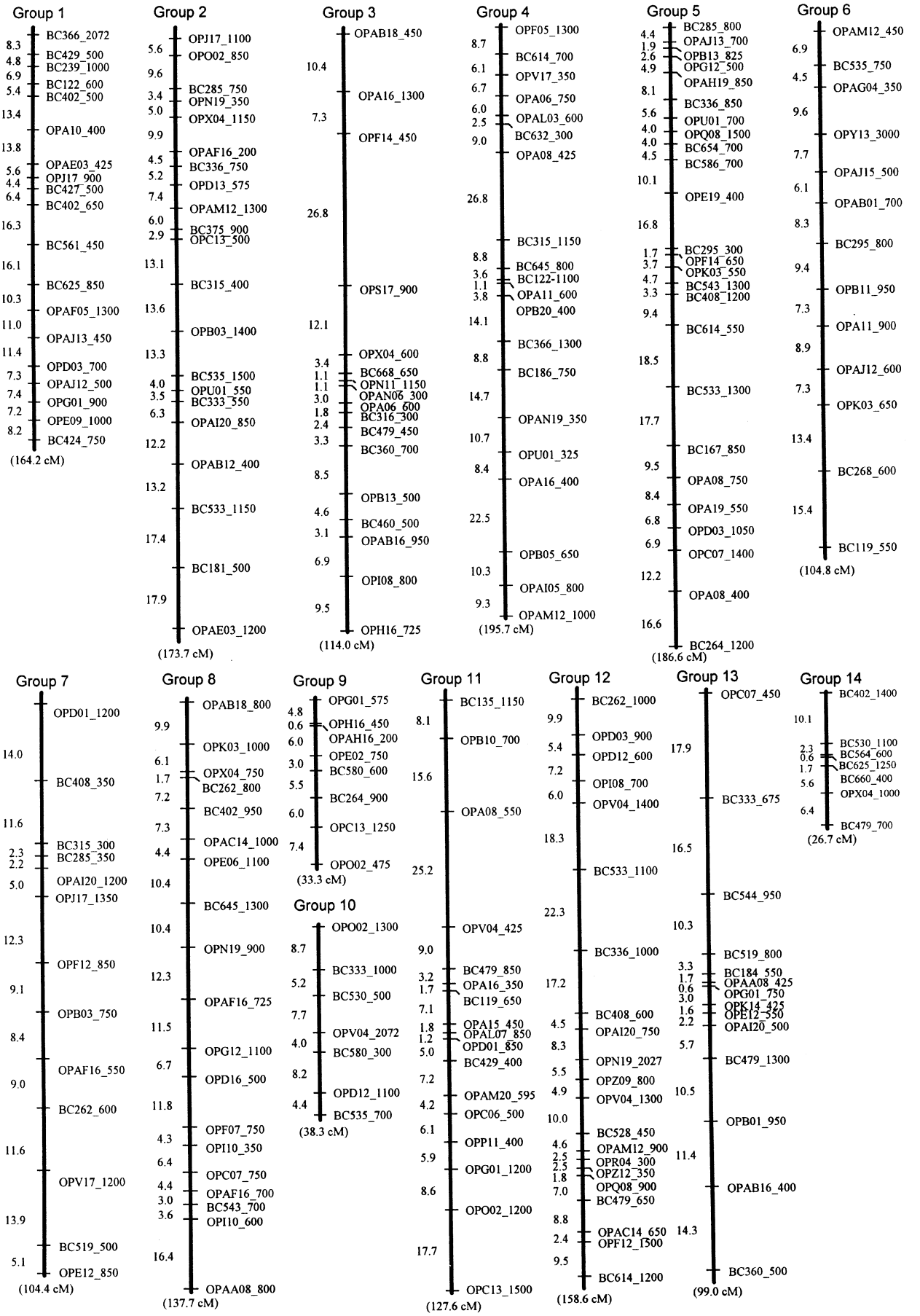
## Results

### Linkage-map construction

From the 132 selected primers, 267 segregating RAPD markers were scored on the mapping population. At a LOD threshold for linkage of 4.0, linkage analysis with JOINMAP 2.0 assigned 262 of the markers to 14 linkage groups, with five unassigned. In general, polymorphic loci identified by the same primer were assigned to different linkage groups. However, when such markers co-segregated, i.e. when markers amplified by the same primer showed little or no recombination with one another, they were considered to be putative co-dominants. Seven pairs of markers were observed to be co-segregants, and only one of such pairs was retained in the dataset. To check for genotyping errors, the JOINMAP module for genotype checking was used to calculate, for all loci, the probability of obtaining the present genotype, conditional on both the genotypes at the two flanking loci and on map distances. We considered loci with a threshold of $\geq 3$ for the test statistic as having improbable genotypes. Such loci were deleted from the data file, and the linkage maps re-calculated. The genotype checking was continued until the linkage groups had their loci showing a test statistic of less than 2.0.

The data files were then imported into MAPMANAGER (Manly and Cudmore 1994) to check further for possible genotyping errors. The final maps (Fig. 1) were constructed with 222 markers and

**Fig. 1** RAPD-based genetic linkage maps of radiata pine consisting of 222 markers and constructed using JOINMAP and MAPMANAGER softwares. Segregation data were obtained from haploid DNA of 93 megagametophytes. Marker identification, given on the right of the bars, follows a standard convention with the 'OP' and 'BC' prefixes indicating primer source and with fragment size given in base-pairs; interval distances (in centiMorgans, cM) are indicated to the left of the bars


Group 1
BC366_2072
8.3
BC429_500
4.8
BC239_1000
6.9
BC122_600
5.4
BC402_500
13.4
OPA10_400
13.8
OPAE03_425
5.6
OPJ17_900
4.4
BC427_500
6.4
BC402_650
16.3
BC561_450
16.1
BC625_850
10.3
OPAF05_1300
11.0
OPAJ13_450
11.4
OPD03_700
7.3
OPAJ12_500
7.4
OPG01_900
7.2
OPE09_1000
8.2
BC424_750
(164.2 cM)
Group 2
OPJ17_1100
5.6
OPO02_850
9.6
BC285_750
3.4
OPN19_350
5.0
OPX04_1150
9.9
OPAF16_200
4.5
BC336_750
5.2
OPD13_575
7.4
OPAM12_1300
6.0
BC375_900
2.9
OPC13_500
13.1
BC315_400
13.6
OPB03_1400
13.3
BC535_1500
4.0
OPU01_550
3.5
BC333_550
6.3
OPAI20_850
12.2
OPAB12_400
13.2
BC533_1150
17.4
BC181_500
17.9
OPAE03_1200
(173.7 cM)
Group 3
OPAB18_450
10.4
OPA16_1300
7.3
OPF14_450
26.8
OPS17_900
12.1
OPX04_600
3.4
BC668_650
1.1
OPN11_1150
1.1
OPAN06_300
3.0
OPA06_600
1.8
BC316_300
2.4
BC479_450
3.3
BC360_700
8.5
OPB13_500
4.6
BC460_500
3.1
OPAB16_950
6.9
OPI08_800
9.5
OPH16_725
(114.0 cM)
Group 4
OPF05_1300
8.7
BC614_700
6.1
OPV17_350
6.7
OPA06_750
6.0
OPAL03_600
2.5
BC632_300
9.0
OPA08_425
26.8
BC315_1150
8.8
BC645_800
3.6
BC122-1100
1.1
OPA11_600
3.8
OPB20_400
14.1
BC366_1300
8.8
BC186_750
14.7
OPAN19_350
10.7
OPU01_325
8.4
OPA16_400
22.5
OPB05_650
10.3
OPAI05_800
9.3
OPAM12_1000
(195.7 cM)
Group 5
BC285_800
4.4
OPAJ13_700
1.9
OPB13_825
2.6
OPG12_500
4.9
OPAH19_850
8.1
BC336_850
5.6
OPU01_700
4.0
OPQ08_1500
4.0
BC654_700
4.5
BC586_700
10.1
OPE19_400
16.8
BC295_300
1.7
OPF14_650
3.7
OPK03_550
4.7
BC543_1300
3.3
BC408_1200
9.4
BC614_550
18.5
BC533_1300
17.7
BC167_850
9.5
OPA08_750
8.4
OPA19_550
6.8
OPD03_1050
6.9
OPC07_1400
12.2
OPA08_400
16.6
BC264_1200
(186.6 cM)
Group 6
OPAM12_450
6.9
BC535_750
4.5
OPAG04_350
9.6
OPY13_3000
7.7
OPAJ15_500
6.1
OPAB01_700
8.3
BC295_800
9.4
OPB11_950
7.3
OPA11_900
8.9
OPAJ12_600
7.3
OPK03_650
13.4
BC268_600
15.4
BC119_550
(104.8 cM)
Group 7
OPD01_1200
14.0
BC408_350
11.6
BC315_300
2.3
BC285_350
2.2
OPAI20_1200
5.0
OPJ17_1350
12.3
OPF12_850
9.1
OPB03_750
8.4
OPAF16_550
9.0
BC262_600
11.6
OPV17_1200
13.9
BC519_500
5.1
OPE12_850
(104.4 cM)
Group 8
OPAB18_800
9.9
OPK03_1000
6.1
OPX04_750
1.7
BC262_800
7.2
BC402_950
7.3
OPAC14_1000
4.4
OPE06_1100
10.4
BC645_1300
10.4
OPN19_900
12.3
OPAF16_725
11.5
OPG12_1100
6.7
OPD16_500
11.8
OPF07_750
4.3
OPI10_350
6.4
OPC07_750
4.4
OPAF16_700
3.0
BC543_700
3.6
OPI10_600
16.4
OPAA08_800
(137.7 cM)
Group 9
OPG01_575
4.8
OPH16_450
0.6
OPAH16_200
6.0
OPE02_750
3.0
BC580_600
5.5
BC264_900
6.0
OPC13_1250
7.4
OPO02_475
(33.3 cM)
Group 10
OPO02_1300
8.7
BC333_1000
5.2
BC530_500
7.7
OPV04_2072
4.0
BC580_300
8.2
OPD12_1100
4.4
BC535_700
(38.3 cM)
Group 11
BC135_1150
8.1
OPB10_700
15.6
OPA08_550
25.2
OPV04_425
9.0
BC479_850
3.2
OPA16_350
1.7
BC119_650
7.1
OPA15_450
1.8
OPAL07_850
1.2
OPD01_850
5.0
BC429_400
7.2
OPAM20_595
4.2
OPC06_500
6.1
OPP11_400
5.9
OPG01_1200
8.6
OPO02_1200
17.7
OPC13_1500
(127.6 cM)
Group 12
BC262_1000
9.9
OPD03_900
5.4
OPD12_600
7.2
OPI08_700
6.0
OPV04_1400
18.3
BC533_1100
22.3
BC336_1000
17.2
BC408_600
4.5
OPAI20_750
8.3
OPN19_2027
5.5
OPZ09_800
4.9
OPV04_1300
10.0
BC528_450
4.6
OPAM12_900
2.5
OPR04_300
2.5
OPZ12_350
1.8
OPQ08_900
7.0
BC479_650
8.8
OPAC14_650
2.4
OPF12_1300
9.5
BC614_1200
(158.6 cM)
Group 13
OPC07_450
17.9
BC333_675
16.5
BC544_950
10.3
BC519_800
3.3
BC184_550
1.7
OPAA08_425
0.6
OPG01_750
3.0
OPK14_425
1.6
OPE12_550
2.2
OPAI20_500
5.7
BC479_1300
10.5
OPB01_950
11.4
OPAB16_400
14.3
BC360_500
(99.0 cM)
Group 14
BC402_1400
10.1
BC530_1100
2.3
BC564_600
0.6
BC625_1250
1.7
BC660_400
5.6
OPX04_1000
6.4
BC479_700
(26.7 cM)

covered a total distance of 1665 cM. The maps provided a resolution of one marker every 8 cM, on average. The number of markers per linkage group ranged from 7 to 25, while the proportion of missing genotypes per linkage group ranged from 2.9% to 6.7%.

The extent of genome coverage was determined by first estimating the expected total length of the radiata pine genome using "method 3" of Chakravarti et al. (1991). The largest observed map distance between linked markers at a LOD score of $\geq 4.0$ was 26.8 cM. The observed number of locus pairs with a LOD score of 4.0 or greater (K) was 735. These values were used to calculate the expected length of the radiata pine genome as 1789 cM. Thus the current set of 222 loci is apparently distributed over approximately 93% of the genome.

## Age–age variation in stem growth

The means and coefficients of variation for age-specific measurements made at various stages of radiata pine seedling growth are presented in Table 1. Although observed phenotypic variations (as determined by the coefficients of variation) for diameter and height were not large, they were substantial for stem volume. Normality tests for all traits showed no evidence of a significant departure from a normal distribution, except for stem volume at 5 months of age. The data was log-transformed to improve normality, but results from QTL analysis using the transformed data did not differ from those with untransformed data. Therefore, only untransformed data were used.

## QTL dissection of stem growth variation

The total number of putative QTLs detected for stem growth variables ranged from 12 for stem volume to 21 for diameter, and were spread across 10 of the 14 linkage groups in our linkage map (Table 2). The explained variation associated with significant QTLs ranged from 6.8% to 30.0%. Most of the high percentage effects were associated with QTLs affecting stem volume, suggesting that major QTLs might be segregating for this trait in the mapping population. Across traits, the highest number of putative QTLs was detected for the first-year growth traits and the least for the third-year measurements.

We observed 31 age–age QTL trends in which at least one significant LOD score was observed across the four ages considered for all traits (height, diameter and volume). None of the 31 trends showed complete stability, i.e. none of the putative QTL positions detected at any one age was strongly expressed at all of the four stages of measurement (5 months, 1, 2 and 3 years after germination). However, 45% of the trends showed partial stability, i.e. putative QTLs significant at one age were also detected at a subsequent age. The observed trend in QTL expression with age can be broadly classified into three categories, which are summarised as follows (Fig. 2).

(1) QTLs at some locations showed a gradual linear increase in influence from 5 months of age, the LOD peaks and explained variation showing a linear trend in magnitude as the seedlings aged and were highest at 3 years of age ('A' in Fig. 2; Table 2). This trend can be most aptly illustrated for a putative QTL detected for diameter and volume in linkage group 1 (Fig. 3). For both traits, the LOD peaks at the QTL position closest to marker BC239_1000 showed a gradual increase from 0.02 for measurements taken at 5 months of age to a maximal value of 6.4–7.7 at 3 years of age. This pattern of QTL expression was the least common and was encountered only four times in a total of 31 trends observed across all traits.

**Table 1** Summary of the distribution statistics for measurements made at various stages of growth on 93 seedlings of radiata pine from a full-sib cross

| Traits | Min. | Max. | Mean | CV%[a] | KS distance[b] | *P*-value |
|---|---|---|---|---|---|---|
| Diameter | | | | | | |
| 5 months (mm) | 1.88 | 4.77 | 3.64 | 17.35 | 0.14 | 0.06 |
| 1 year (mm) | 8.45 | 32.95 | 22.89 | 21.64 | 0.05 | > 0.10 |
| 2 years (mm) | 19.21 | 53.40 | 38.42 | 16.71 | 0.05 | > 0.10 |
| 3 years (mm) | 14.99 | 91.49 | 67.31 | 16.82 | 0.07 | > 0.10 |
| Volume | | | | | | |
| 5 months (cm3) | 0.20 | 5.57 | 2.74 | 48.52 | 0.15 | 0.02* |
| 1 year (m3) | 0.02 | 1.02 | 0.41 | 50.81 | 0.08 | > 0.10 |
| 2 years (m3) | 0.21 | 4.75 | 2.08 | 43.61 | 0.10 | > 0.10 |
| 3 years (m3) | 0.31 | 6.86 | 3.29 | 42.75 | 0.06 | > 0.10 |
| Height | | | | | | |
| 5 months (cm) | 7.00 | 31.20 | 23.6 | 22.90 | 0.10 | > 0.10 |
| 1 year (m) | 0.34 | 1.24 | 0.91 | 16.83 | 0.08 | > 0.10 |
| 2 years (m) | 0.71 | 2.30 | 1.68 | 15.79 | 0.08 | > 0.10 |
| 3 years (m) | 0.61 | 3.42 | 2.55 | 15.20 | 0.06 | > 0.10 |

[a] Coefficient of variation (%)
[b] Kolmogorov–Smirnov distance statistic

**Table 2** Biometrical parameters of detected QTLs affecting seedling growth at 5 months, 1, 2 and 3 years of age in the radiata pine family 30040 × 80121

| Trait | Linkage group | Closest marker | Peak LOD | | | | Observed trend[a] |
|---|---|---|---|---|---|---|---|
| | | | 5 months | 1 year | 2 years | 3 years | |
| Diameter | 1 | BC239_1000 | 0.02 | 2.40 | 5.24 | 6.37 | A |
| | 2 | OPB03_1400 | 2.63 | 0.51 | 1.04 | 0.86 | B |
| | 2 | BC533_1150 | 4.12 | 3.11 | 0.36 | 0.21 | B |
| | 3 | OPAB18_450 | 0.21 | 0.01 | 1.12 | 2.88 | A |
| | 3 | OPX04_600 | 2.91 | 0.36 | 0.41 | 0.42 | B |
| | 3 | BC460_500 | 0.31 | 2.79 | 0.20 | 0.71 | C |
| | 4 | OPA11_600 | 0.16 | 3.39 | 0.49 | 1.78 | C |
| | 6 | BC295_300 | 3.34 | 2.68 | 0.45 | 0.33 | B |
| | 6 | BC268_600 | 3.54 | 0.82 | 0.23 | 0.01 | B |
| | 8 | OPAC14_1000 | 0.00 | 0.27 | 3.77 | 0.26 | C |
| | 9 | BC580_600 | 0.10 | 3.35 | 2.65 | 0.53 | C |
| | 11 | OPO02_1200 | 0.03 | 3.26 | 5.78 | 3.10 | C |
| | 13 | OPB01_950 | 1.38 | 3.03 | 2.81 | 2.29 | C |
| | 14 | OPX04_1000 | 0.10 | 0.06 | 4.16 | 0.98 | C |
| No. of QTLs | | | 5 | 7 | 6 | 3 | |
| Volume | 1 | BC239_1000 | 0.02 | 0.35 | 2.45 | 7.65 | A |
| | 6 | OPA11_900 | 7.89 | 5.17 | 0.58 | 1.02 | B |
| | 6 | BC268_600 | 4.50 | 1.57 | 0.07 | 0.05 | B |
| | 9 | OPE02_750 | 0.23 | 6.45 | 0.69 | 1.83 | C |
| | 11 | OPO02_1200 | 0.01 | 4.60 | 5.90 | 4.19 | C |
| | 12 | BC533_1100 | 0.02 | 0.25 | 0.48 | 2.62 | A |
| | 13 | OPB01_950 | 0.00 | 5.08 | 4.64 | 2.70 | C |
| No. of QTLs | | | 2 | 4 | 2 | 4 | |
| Height | 1 | BC366_2072 | 0.97 | 3.33 | 0.48 | 0.78 | C |
| | 1 | BC625_850 | 2.52 | 1.13 | 0.33 | 0.09 | B |
| | 2 | OPB03_1400 | 2.85 | 0.33 | 0.8 | 0.70 | B |
| | 2 | BC181_700 | 3.63 | 0.73 | 0.01 | 1.20 | B |
| | 6 | OPB11_950 | 5.44 | 4.07 | 4.12 | 0.82 | B |
| | 7 | BC408_350 | 0.57 | 3.27 | 3.94 | 0.08 | C |
| | 9 | OPE02_750 | 1.29 | 3.77 | 0.76 | 0.54 | C |
| | 11 | OPO021200 | 0.00 | 2.72 | 3.91 | 0.76 | C |
| | 12 | OPZ09_800 | 1.07 | 3.50 | 3.83 | 2.09 | C |
| | 13 | BC519_800 | 3.02 | 0.18 | 1.45 | 0.33 | B |
| No. of QTLs | | | 5 | 6 | 4 | 0 | |

[a] See text for explanation of the $A^-$, $B^-$ and C-type trends

For height measurements, the LOD profile in linkage group 1 suggested a different pattern of age–age QTL expression (Fig. 3). Instead of a gradual increase in expression of a single QTL with age, a shift in location to adjacent intervals was observed for putative QTLs affecting height at the various stages of growth considered.

(2) A common trend observed in age–age QTL expressions involved putative QTLs detected at 5 months of age that gradually became less significant as the seedlings aged, and no longer showed any influence on the character at 3 years of age ('B' in Fig. 2; Table 2). Twelve of the thirty one age–age trends in QTL expression fit this pattern, which is best illustrated for a region in linkage group 6 (Fig. 3). For all three traits, the LOD scores were maximal at 5 months of age, ranging from 3.3 to 7.9. At 1 year of age, some peak LOD scores at these locations were still significant, but at 2 years only the peak for height was significant and at 3 years the LOD scores had dropped to a mean value of 0.4.

(3) Another common trend involved some putative QTLs which showed a gradual curvilinear increase in effect from 5 months of age, reaching their peak expression at 1 to 2 years, and sometimes were still detected at 3 years of age ('C' in Fig. 2; Table 2). The LOD profiles obtained for putative QTLs affecting diameter and volume in linkage groups 11 and 13 demonstrate this observed pattern (Fig. 4). In the two linkage groups, the peak LOD scores for diameter and volume at 5 months of age were not significant. However, at 1, 2 and 3 years of age, the peak LOD scores at the same genomic locations exceeded the threshold value of 2.5, indicating a partially stable expression of the putative QTLs. For height measurements, the peak LOD scores were stable

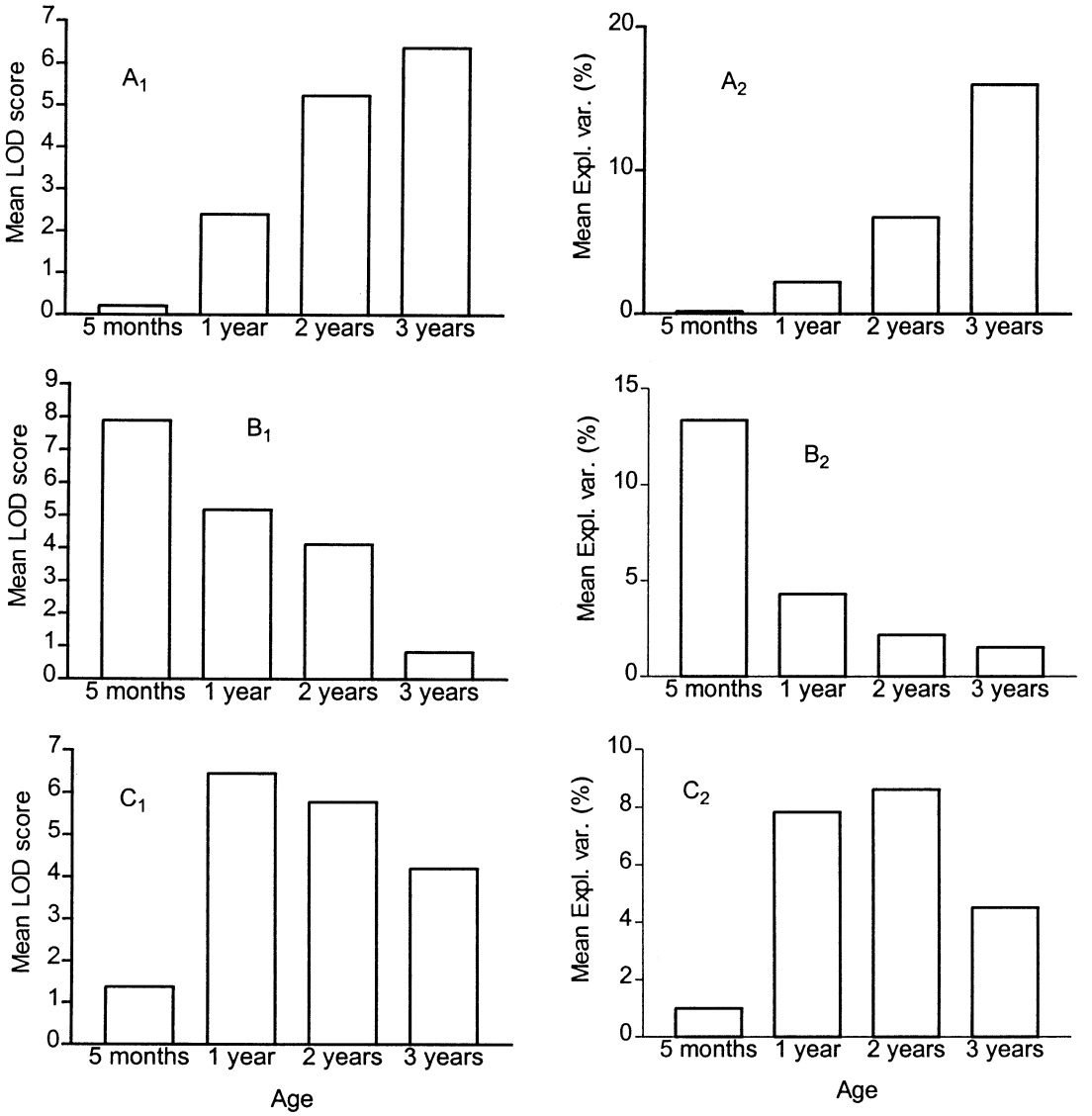



**Fig. 2** Graph of the observed pattern of QTL expression (A–C) using mean value of LOD scores ($A_1$–$C_1$) and the explained variation ($A_2$–$C_2$) of putative QTLs detected for all traits (height, diameter and volume) measured at various growth stages (time from germination)

for the first 2 years, but were below the threshold value at 3 years of age.

## Discussion

Although changes in the genetic control of growth in forest trees have been studied in the past using quantitative genetic methods (Namkoong et al. 1972; Matheson et al. 1994), traditional heritability estimates represent the cumulative effects of all genes (Vasquez and Dvorak 1996) and say nothing about any particular gene. However, there is a surprising degree of consensus among researchers on the genetic basis of growth. For example, Kremer (1992) proposed that a given set of genes affect height growth and is progressively modified: after each season a portion of the set is replaced and, several years later, the original set has been totally modified. Hodge and White (1992) gave the same genetic interpretation in a synthetic study of many progeny tests of slash pine (*Pinus elliotti* Engelm. var. *elliottii*) measured at different ages. Genetic factors controlling height growth could be the same, but depending on annual conditions and the age of the trees the expression of these genes would be variable. Costa and Durel (1996) are of the view that growth could be determined by a more constant set of genes whose expression is only slightly modified during the course of tree development.

Using the methods of quantitative trait locus analysis, this study demonstrated variable expression of stem

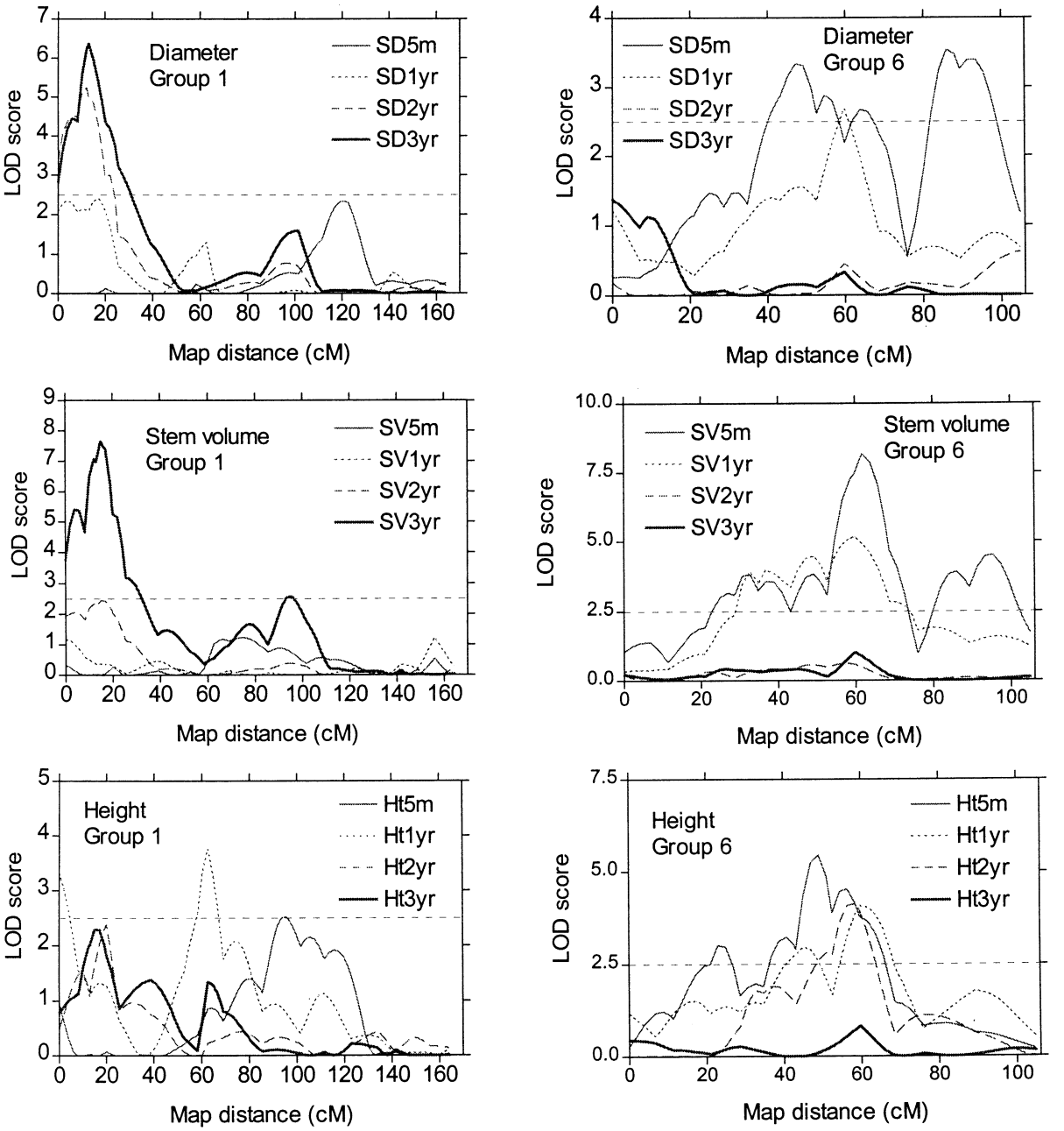



**Fig. 3** LOD score profile of linkage groups 1 and 6 for stem growth measurements made over a 3-year period. SD5m, Ht5m and SV5m represent LOD plots of measurements on diameter, volume and height made at 5 months of age. Plots for the 1-, 2- and 3-years measurements are similarly indicated. The *dotted horizontal lines* indicate the 5% significance threshold

growth QTLs during the early vegetative growth period of radiata pine. Our results showed conformity with the suggestion of Atchley (1984) that growth variations may result from the activation and repression of genes responsible for changes in growth. The number of putative QTLs detected for any of the measured traits varied with age, and none of the QTLs detected at any one age was strongly expressed at all of the four stages of measurement. Across all putative loci and traits, half of the age–age QTL trends followed a curvilinear pattern, while loci in the other half showed a linear increase or decrease in influence. Thus, superimposed on the age–age QTL trends, outlined above in the Results section, was a general pattern of QTL activation and repression throughout the period of study, suggesting that genes were being switched on and off.

We also observed an interesting pattern of QTL activation-repression for height in linkage group 1 where there was a progressive shift to adjacent intervals involving putative QTLs detected at different ages (Fig. 3). The region involved probably contained

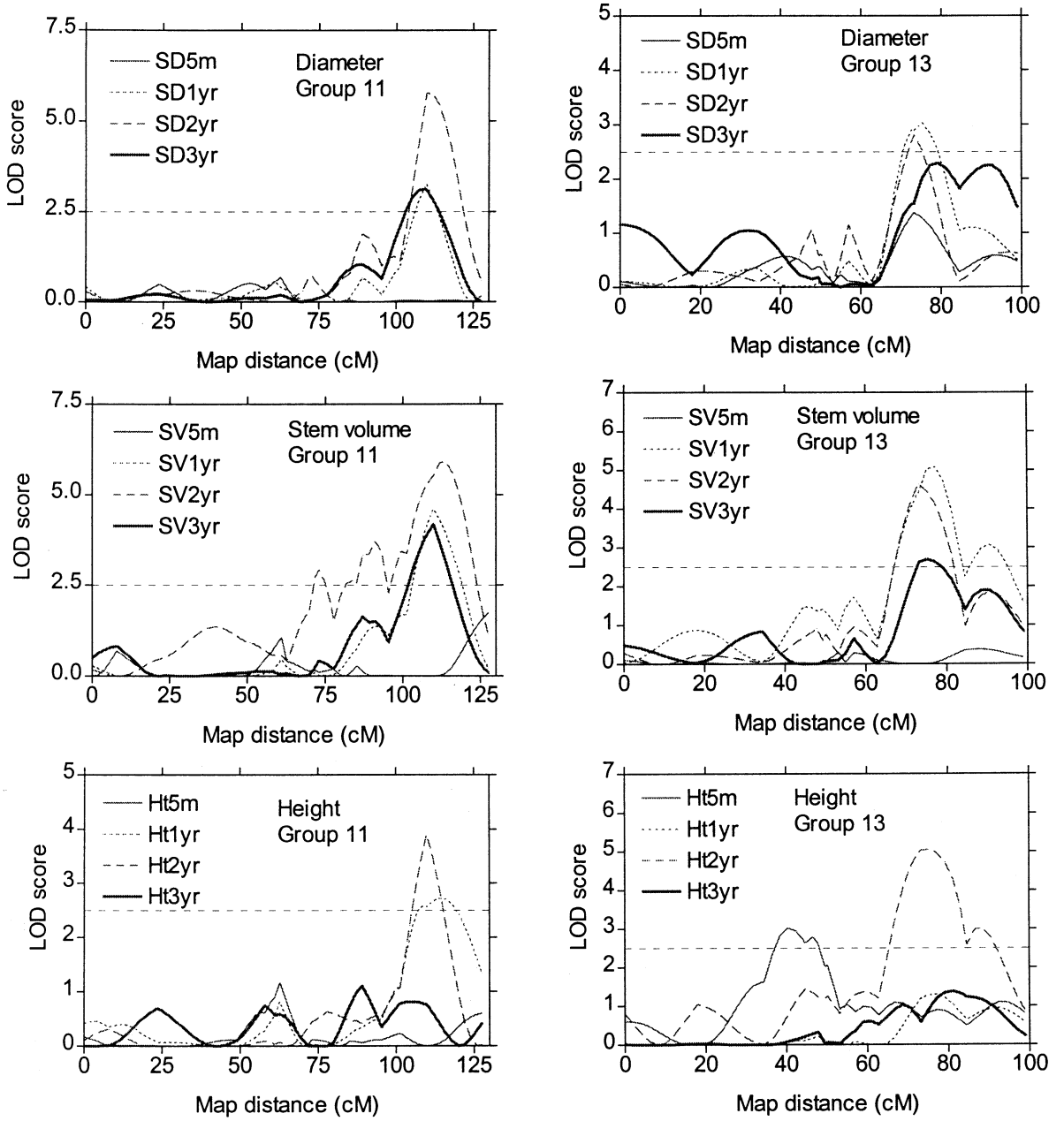



**Fig. 4** LOD score profile of linkage groups 11 and 13 for stem growth measurements made over a 3-year period. SD5m, Ht5m and SV5m represent LOD plots of measurements on diameter, volume and height made at 5 months of age. Plots for the 1-, 2- and 3-years measurements are similarly indicated. The *dotted horizontal lines* indicate the 5% significance threshold

a cluster of QTLs for height and, as the genome faced changes in the environment over the study period, some loci were repressed while new ones were activated to deal with these changes. In contrast, LOD scores obtained for diameter and volume in the linkage group did not show any evidence of a progressive shift along a genomic region (Fig. 3). Rather than a switch in QTL position, there was a progressive temporal change in the phenotypic effect of a single QTL residing at the same location.

It might be tempting to suggest from our results that age–age variation in trees arises from the differential expression of stem growth QTLs. However, this would be premature at this stage because we have considered only the first 3 years of a tree's lifetime. The main environmental influences upon seedling growth at this stage are microclimate and micro-site soil variable factors. It was not possible to determine whether the differential expression of stem growth QTLs at this stage is related to maturational effects or to the differential ability to adapt to a new environment. Moreover,

marker-locus × age interaction could result from residual variations caused by non-genetic factors, which differ largely over years of growth. The magnitude of the non-genetic variation directly influences the explained variance of a QTL, and thus its LOD score (Ooijen 1992). Measurement errors, as well as the number of replications (in this case, the size of the mapping population), influence residual non-genetic factors.

Our results, however, agree well with those obtained for other perennial plant species. Verhaegen et al. (1997) reported recently that, in a study with *Eucalyptus*, no QTLs affecting growth and wood properties were significant across three ages of trait measurement. Similarly, Plomion et al. (1996) reported that in a study done with maritime pine, none of the QTLs significant at one stage of height growth were also significant at another stage. Asins et al. (1994) showed that only 3 out of 17 detected QTLs affecting morphological traits in almond were homogenous across 3 years of growth. Sondur et al. (1995) found QTLs affecting growth in papaya (*Carica papaya* L.) to be developmentally sensitive, influencing growth during different seasons.

Given the importance of QTL stability for marker-aided selection, an understanding of the pattern and stability of QTL expression in the course of tree growth is essential. The field trial used for this study may be re-measured over the years to provide additional insights on the pattern and stability of stem-growth QTLs.

**Acknowledgments** Grants from the Southern Tree Breeding Association (STBA), Australia, and the Australian National University, Canberra, supported this research. We thank Drs. J. Glaubitz and C. Bell for reviewing the initial draft of the manuscript, as well as two anonymous reviewers for their valuable comments.

## References

Asins MJ, Mestre P, Garcia JE, Dicenta, F, Carbonell EA (1994) Genotype × environment interaction in QTL analysis of an intervarietal almond cross by means of genetic markers. Theor Appl Genet 89 : 258–264

Atchley WR (1984) Ontogeny, timing of development, and genetic variance-covariance structure. Am Nat 123 : 519–540

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, Stettler RF (1995) Molecular genetics of growth and development in *Populus*. IV. Mapping QTL with large effects on growth, form, and phenology in a forest tree. Genetics 139 : 963–973

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

Chakravarti A, Lasher LA, Reefer JE (1991) A maximum-likelihood method for estimating genome length using genetic linkage data. Genetics 128 : 175–182

Costa P, Durel CE (1996) Time trends in genetic control over height and diameter in Maritime pine. Can J For Res 26 : 1209–1217

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

Emebiri LC, Devey MI, 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

Grattapaglia D, Bertolucci FLG, Penchel R, Sederoff RR (1996) Genetic mapping of quantitative trait loci controlling growth and wood quality traits in *Eucalyptus grandis* using a maternal half-sib family and RAPD markers. Genetics 144 : 1205–1214

Greenwood MS, Volkeart HA (1992) Morphological traits as markers for the early selection of conifer genetic families. Can J For Res 22 : 1001–1008

Hodge GR, White TL (1992) Genetic parameter estimates for growth at different ages in slash pine and some implications for breeding. Silvae Genet 41 : 252–261

Hutchinson KW, Greenwood MS (1991) Molecular approaches to gene expression during conifer development and maturation. For Ecol Management 43 : 273–286

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

Kremer A (1992) Prediction of age–age correlations of total height based on serial correlations between height increments in maritime pine (*Pinus pinaster* Ait.). Theor Appl Genet 85 : 152–158

Lander ES, Botstein D (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121 : 185–199

Manly K, Cudmore R (1994) MapManager. A program for genetic mapping. Roswell park Cancer Institute, New York

Matheson AC, Spencer DJ, Magnussen D (1994) Optimum age for selection in *Pinus radiata* using basal area under bark for age:age correlations. Silvae Genet 43 : 352–357

Namkoong G, Usanis RA, Silen RR (1972) Age-related variation in genetic control of height growth in Douglas-fir. Theor Appl Genet 42 : 151–159

Ooijen JW (1992) Accuracy of mapping quantitative trait loci in autogamous species. Theor Appl Genet 84 : 803–811

Ooijen JW, Maliepaard C (1996) MAPQTL version 3.0 : software for calculation of QTL positions on genetic maps. DLO-CPRO, Wageningen

Plomion C, Durel CE, O'Malley DM (1996) Genetic dissection of plant height in maritime pine seedlings raised under accelerated growth conditions. Theor Appl Genet 93 : 849–858

Sondur SN, Manshardt RM, Stiles JI (1995) Genetics of growth rate and flowering time in Papaya (*Carica papaya* L.). Journal of Agricultural Genomics, vol 1: (http://www.ncgr.org/ag/jag/index.html)

Stam S, Ooijen JW (1995) JOINMAP version 2.0. Software for the calculation of genetic linkage maps, CPRO-DLO, Wageningen.

Vasquez J, Dvorak WS (1996) Trends in variances and heritabilities with stand development of tropical pines. Can J For Res 26 : 1473–1480

Verhaegen D, Plomion C, Gion J-M, Poitel M, Costa P, Kremer A (1997) Quantitative trait dissection analysis in *Eucalyptus* using RAPD markers. 1. Detection of QTLs in interspecific hybrid progeny, stability of QTL expression across different ages. Theor Appl Genet 95 : 597–608

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, Tungey 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