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Quantitative trait dissection analysis in *Eucalyptus* using RAPD markers: 1. Detection of QTL in interspecific hybrid progeny, stability of QTL expression across different ages

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Abstract The objective of this study was to use random amplified polymorphic DNA (RAPD) to determine the genetic location and effects of genomic regions controlling wood density, stem growth and stem form in two species of *Eucalyptus*. Two hundred F_1 trees generated from an interspecific cross *E. urophylla* × *E. grandis* between two elite trees were used. Genetic maps were constructed for each parent with markers segregating in the 1:1 ratio in FS progeny. A total of 86 and 92 markers distributed among 11 linkage groups covered 1295 cM and 1312 cM for the *E. urophylla* and *E. grandis* parent, respectively. Traits were measured three times up to selection age (38 months). The magnitude of the phenotypic variation explained by the joint action of the segregating quantitative trait alleles indicated that genetic factors of large effect were involved in the control of the studied characters. Several regions controlling part of the variation for the studied traits were identified by interval mapping. Some regions of the genome exerted effects on more than one trait, providing a genetic explanation for at least some of the correlation between the traits. On the basis of an age-by-age analysis, a partial stability of QTL expression was observed with 68% of the QTL being expressed at two ages and 32% being age-specific. No QTL were significant for all three ages. Taking advantage of repeated measurements on the same material across different ages, we investigated with a maximum statistical power, the effect of marker genotype on traits, with age and QTL × age interaction effects being removed. A two-way analysis of variance made it possible to detect significant marker-trait associations over the period studied. Most of them had already been detected in the annual analysis. This result is very encouraging for the application of marker information to the early selection of hybrid trees to be vegetatively propagated for the production of clonal varieties.

Key words *Eucalyptus* · Genetic mapping · QTL · Stability · Marker-assisted selection

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Introduction

Tree improvement is limited by the time needed to reach sexual maturity and the time lag required to evaluate growth performances (McKeand 1988; Cotterill and Dean 1988; Kremer 1992; Hodge and White 1992; Danjon 1994). In addition, tree selection remains imprecise because environmental effects are rather high for the major economic traits. Heritabilities for height, diameter, volume, branching traits and straightness are in the range of 0.1–0.3 and are only slightly higher for the specific gravity of wood (Cornelius 1993). In that context, any tool directed toward a selection procedure that improves the evaluation of genetic value and reduces the generation time would be of considerable value.

Individual loci controlling the variation of quantitative traits (QTL: quantitative trait loci) have been detected and mapped on the genome of many plant species (reviewed by Tanksley 1993; Paterson 1995). The manipulation of marker-QTL information has shown itself to be promising for increasing the selection efficiency (Lande and Thompson 1990). In crop plants, QTL mapping is usually performed within a single pedigree and therefore explores the within-family linkage disequilibrium between marker and QTL alleles. F_2s (e.g. Edwards et al. 1987; Paterson et al. 1991), recombinant inbred lines (e.g. Goldman et al. 1995),

double haploid lines (e.g. Hyne et al. 1994; Ferreira et al. 1995) and backcross progenies (e.g. Komatsuda et al. 1993) of relatively large size (from 100 to 1000) are widely used in these species. Such pedigrees are not available in most forest trees because inbreeding depression is usually high in these allogamous species or because haploidization technology has not yet been developped. Thus, phenotypic variation is generally associated with genetic markers in two- to three-generation outbred pedigrees, either full-sib (Groover et al. 1994; Bradshaw and Stettler 1995; Grattapaglia et al. 1995) or half-sib families (Grattapaglia et al. 1996; O'Malley et al. 1994).

Under non-optimal conditions for major species, interspecific hybrids of *Eucalyptus grandis* × *Eucalyptus urophylla* are the best available material for commercial plantations in the Congo (Vigneron 1991). This combination offers the good productivity form and easy vegetative propagation of *E. grandis* and the excellent adaptability of *E. urophylla*. A reciprocal recurrent selection (RRS) scheme has been adopted for the improvement of parental populations and for the creation of hybrid clonal varieties. Our first objective was to study the genetics of those traits of commercial value: wood density, stem growth and stem form, by QTL analysis in a single hybrid family. Forest trees are long-lived organisms that can experience a diverse array of environmental conditions during their lifetime. In that context, our second objective was to investigate the stability of putative QTL over time to evaluate the potential of early marker-assisted selection.

We based our study on previously reported genetic maps of *E. urophylla* and *E. grandis* that were constructed with random amplified polymorphic DNA (RAPD) markers (Williams et al. 1990) segregating in the F_1 family studied (Verhaegen and Plomion 1996).

Materials and methods

Genetic material and linkage map construction

An interspecific family between elite *Eucalyptus urophylla* and *E. grandis* trees was used to detect putative QTL. Both parental trees were chosen because they displayed the highest general combining abilities in the different mating designs used in Congo. In addition, the hybrid family resulting from this specific cross was among the best tested in the Congo and is widely used in commercial plantations in that country. Saturated genetic maps of both parental trees and the alignment of homologous linkage groups in both species have been described elsewhere (Verhaegen and Plomion 1996). Those comprise 269 and 236 RAPD markers for the *E. urophylla* and *E. grandis* individual parents, respectively. These two maps were constructed with 93 F_1 hybrids, while for the QTL analysis the sample was expanded to 201 individuals. The additional 108 progeny were genotyped with a subset of RAPD markers in the testcross configuration (1:1 segregation ratio). Procedures for DNA extraction and RAPD assay are given elsewhere (Verhaegen and Plomion 1996). Linkage analysis with the 201 progeny was performed using MAPMAKER (Lander et al. 1987) for Macintosh under the backcross model. Markers were grouped with a LOD of 4.0, and the local order was estimated using an interval support of at least 3.0 (Keats et al. 1991).

Quantitative traits

Assessments of traits were made at three different ages: 18, 26 and 38 months, with 38 months corresponding to half of the rotation (harvest) age in commercial eucalyptus plantations in the Congo. The following traits were measured on the pedigree used for establishing both maps:

- pin penetration depth measured in millimeters with a penetrometer (Pilodyn, PIL). This trait is used in the eucalyptus tree breeding programs to indirectly evaluate wood density: the less the pin enters the trunk, the denser the wood. Density is one of the most important components of the economic value of a pulp-producing plantation of eucalyptus (Petroff and Tissot 1983).
- vigor. Total height, circumference at breast height and volume of the bole were used to determine growth performances of the trees. Since the three traits were highly correlated and gave the same QTL results, the term "vigor" (VIG) was further used to characterize the growth of the bole.
- stem taper (height: diameter ratio, HDR).

Marker-trait associations

While 142 individuals were measured at 18 and 26 months, all trees (201 individuals) were assessed at 38 months. The QTL analysis was performed on each parental map under the backcross model. The rational of such a QTL mapping strategy is described by Plomion and Durel (1996). Linkage groups were scanned every 2 cM using MAPMAKER/QTL (Lincoln and Lander 1990) and QGENE V. 2.26 using the "missing data" option (J.C. Nelson, unpublished software). A $\log_{10}$ of the likelihood odds ratio that a QTL is present versus absent (LOD score) threshold of 2.0 was used to declare a putative QTL. According to the average number of loci and map distances per linkage group, this LOD score for the whole mapping experiment corresponded to a Type-I error rate of approximately 10% (calculated with the program provided by Rebaï; Rebaï et al. 1994). This relatively high Type-I error decreased the Type-II error (probability of not detecting a true effect). The appropriate balance between Type-I and Type-II error rates is not known. The *P* value for type-I error at the LOD peak was calculated from the QGENE software. The "permutation" command of QGENE was further used to ensure the validity of the putative QTL. According to the resampling procedure described by Churchill and Doerge (1994), 1000 random shuffles of the marker data were performed and the 99th percentile of the *F* statistic recorded at the closest RAPD loci flanking the putative QTL. Simultaneous multilocus estimates of the total proportion of phenotypic variation explained by the joint action of the putative QTL were obtained by interval mapping using the "map" command of MAPMAKER/QTL.

Mapping QTL over several years

We performed a QTL search separately for each of the three ages and compared the patterns of QTL expression age by age (annual analysis). However, with observations from multiple ages we could use the fact that repeated observations on the same individuals over different ages were a form of replication which would increase the statistical power for QTL detection (Bradshaw and Foster 1992; Knapp and Bridges 1990). The following two-way analysis of variance (ANOVA) was performed on all loci and ages to investigate the marker, age and marker × age interaction effects:

$$Y_{ijk} = \mu + M_i + A_j + M{*}A_{ij} + \varepsilon_{ijk}$$

where Y_{ijk} is the phenotypic value of individual "k", μ is the overall mean, M_i is the effect of marker "i" (i = presence or absence of a RAPD fragment), A_j is the effect of age "j" (j = 18, 26 or 38 months), $M*A_{ij}$ is the interaction effect between marker "i" and age "j", and ε_{ijk} is the residual. The ANOVA was performed under the SPLUS software. While raw data for PIL and HDR were used as dependent variable in the ANOVA, an incremental rate on a monthly basis within each period (0–18, 18–26 and 26–38) was used for vigor of the stem (VIGrt).

Results

Map construction

A total of 92 and 86 markers were used to rebuild the *E. urophylla* and *E. grandis* maps, respectively (Fig. 1). These markers were amplified from 60 RAPD primers chosen to optimize the genome coverage with the minimal number of RAPD reactions. In addition, most of the selected markers had been previously characterized as framework markers (Verhaegen and Plomion 1996), *i.e.* clearly amplified fragments ordered with a 1000:1 support. A total of 64% and 72% of the markers for the *E. urophylla* and *E. grandis* parent, respectively, were genotyped on 201 individuals, the others (indicated by black dots in Fig. 1) were genotyped on 93 individuals. Eleven linkage groups (LG), corresponding to the haploid number of chromosomes of *Eucalyptus*, were obtained for both maps.

Total map distances of 1295 cM and 1312 cM (Kosambi) were found for *E. urophylla* and *E. grandis*, respectively, i.e. approximately the same sizes as observed in our first mapping experiment (Verhaegen and Plomion 1996). Ordering of the markers was also consistent with the previously reported maps. Thus, despite a twofold increase in sample size (from 93 to 201 for most of the markers), the order of the markers was identical. This result is in favor of the two-step approach proposed by Grattapaglia et al. (1995) for optimizing the intense genotyping work needed in quantitative trait dissection studies, i.e. establish a framework map with a relatively small sample size (between 60 and 90 individuals) and then genotype a whole mapping population using evenly spaced framework markers in order to increase the statistical power for QTL detection (Darvasi et al. 1993).

Detection of QTL in the two parental tree species

Traits important for improving the productivity of the clonal plantations of *Eucalyptus* in the Congo were studied in this QTL mapping experiment. These traits presented a normal distribution. Both of the interval-mapping methods implemented in MAPMAKER/QTL (maximum likelihood) and QGENE (linear least squares) yielded identical results in terms of most likely QTL position and LOD peak value. Table 1 summarizes the main results for QTL detected at 18, 26 and 38 months for the studied traits, and Fig. 1 shows the position and effect of these QTL. Differences in mean trait value between the family mean and the favorable QTL genotype ranged from 0.18σ to 0.46σ, with average values around 0.26σ. We did not detect any significant epistasis. In the following sections the QTL name includes the abbreviation of the trait followed by the linkage group in which this QTL was detected; e.g. PIL18-1 indicates a QTL detected for pilodyn in linkage group 1.

QTL associated with wood density (*PIL*)

QTL associated with PIL were detected for both species and over the three developmental stages (Table 1 and Fig. 1). They accounted for between 5.6% and 10.7% of the total phenotypic variation. For *E. grandis* 4, 3 and 3 QTL were found to be associated with PIL at 18, 26 and 38 months, respectively. Multipoint estimates of the total variation explained by the mapped QTL were 29.7%, 21% and 20.4% at 18, 26 and 38 months, respectively. For *E. urophylla* 1, 2 and 1 QTL accounted for 10.5%, 15.3% and 6% of the phenotypic variation at 18, 26 and 38 months, respectively. In both parental maps, common QTL were detected for successive ages: for *E. grandis*, PIL18-1 and PIL26-1, PIL26-6 and PIL38-6; for *E. urophylla* PIL18-2 and PIL26-2, PIL26-11 and PIL38-11. The other QTL were specific to a single stage.

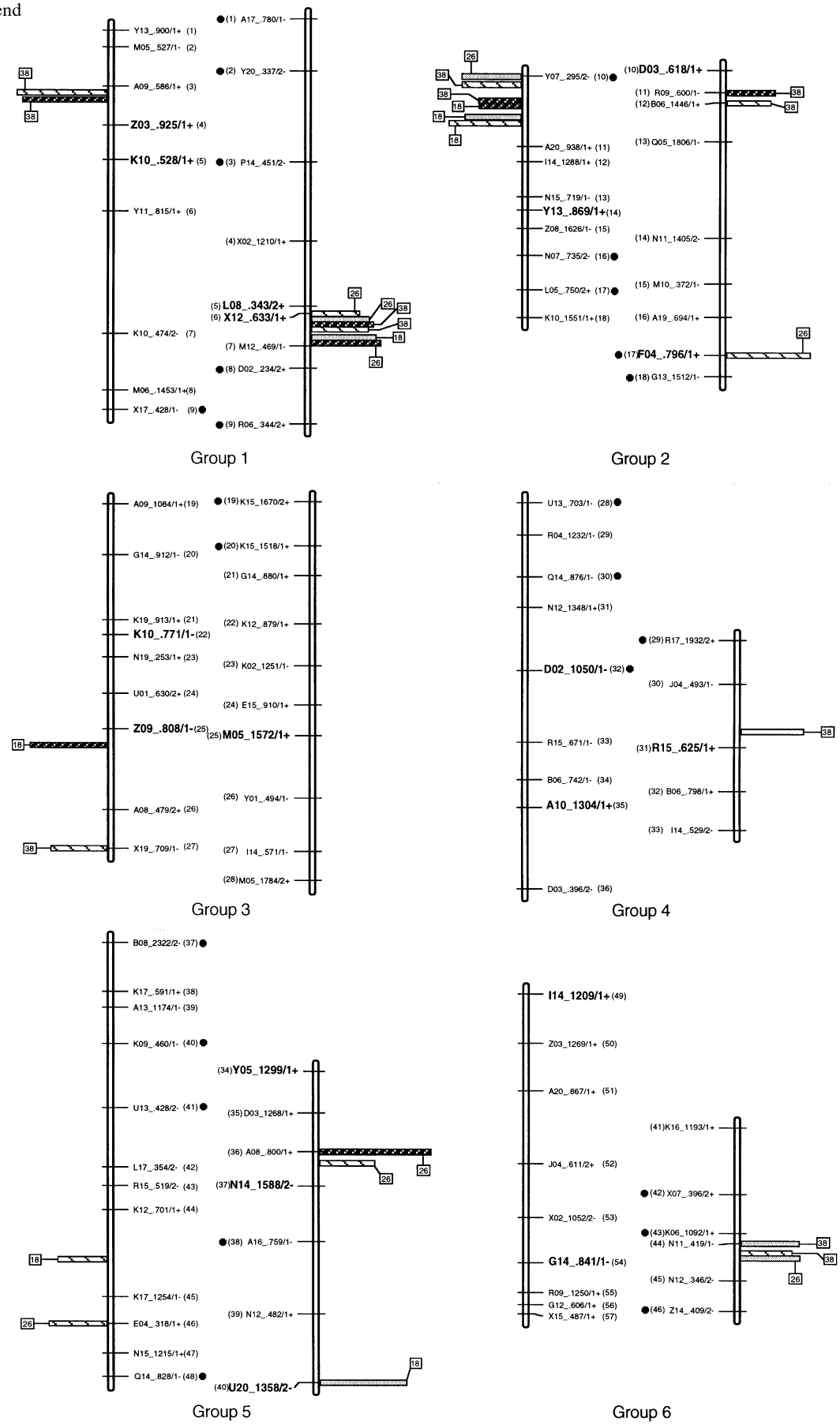
QTL associated with stem form (*height : diameter ratio*, *HDR*)

For both species' maps, individual QTL accounted between 5.1% and 11.0% of the variation. For *E. grandis*, 1, 3 and 2 QTL were detected at 18, 26 and 38 months, respectively. A multilocus model explained 6.2% (HDR18), 23.4% (HDR26) and 15.6% (HDR38) of the trait variation. For *E. urophylla*, 3, 1 and 2 QTL accounted for 25.9% (HDR18), 6.9% (HDR26) and 15.6% (HDR38) of the variation. Again, some QTL were stable over ages: for *E. grandis* HDR18-8 and HDR26-8, HDR26-1 and HDR38-1; for *E. urophylla* HDR18-2 and HDR38-2, and HDR18-11 and HDR26-11. The other QTL were unstable.

QTL associated with stem growth (*VIG*)

Percentage of variation accounted by each growth QTL ranged between 5.1% and 14%. For *E. grandis*, 3 QTL were detected at 26 and 38 months; they explained together 22.0% (VIG26) and 17.7% (VIG38)

Fig. 1 See page 601 for legend



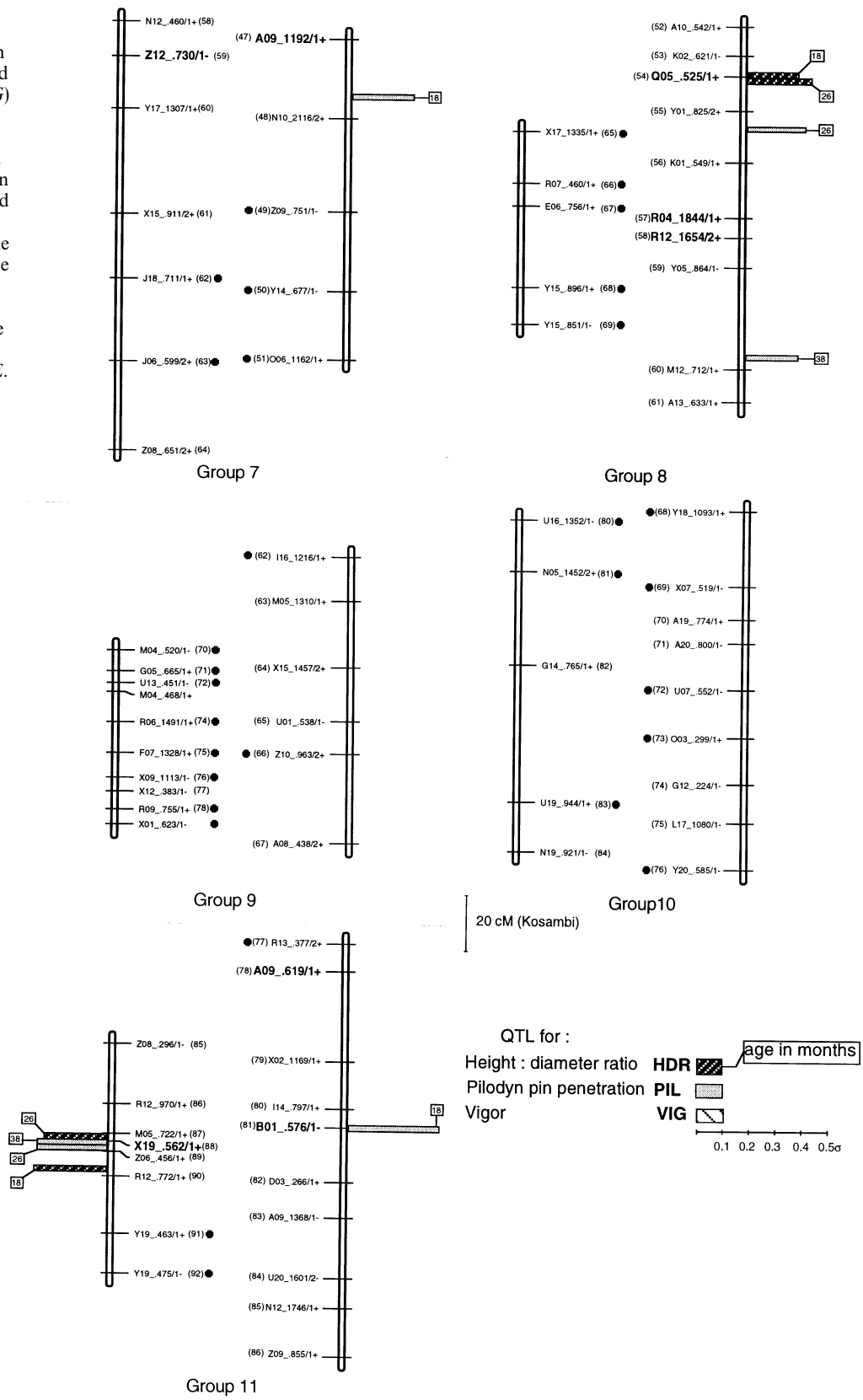


Fig. 1 Genetic maps of *E. urophylla* (*left* map) and *E. grandis* (*right* map) and location of QTL of traits related to wood density (*PIL*), stem growth (*VIG*) and stem form (*HDR*). *Dots* indicate markers assayed on 93 trees, while others were assayed on 201 trees. QTLs are shown in *boxes* with their effects expressed in phenotypic standard deviations (difference between the favorable QTL genotype and the population mean). Age of measurements is also indicated for each putative QTL. Linkage groups of *E. urophylla* were assigned to their homologs in *E. grandis* according to Verhaegen and Plomion (1996)

Table 1 QTL results for *E. urophylla* (A) and for *E. grandis* (B). (*VIG* vigor; *HDR* height : diameter ratio; *PIL* pilodyn pin penetration depth, measured at 18, 26 and 38 months)

A

Trait	Linkage group	Interval	POS[a]	LOD	*P* value	*F*	F99%ile[b]	%var[c]	Δ[d]	R^2m[e]
PIL18	2	10–11	15	2.0	$2.9 \cdot 10^{-3}$	9.2	6.6	10.5	0.23	
PIL26	2	10–11	0	2.3	$1.1 \cdot 10^{-3}$	11.1	6.3	9.2	0.25	
PIL26	11	88–89	0	2.0	$3.0 \cdot 10^{-3}$	9.2	6.6	6.3	0.27	15.3%
PIL38	11	88–89	0	2.7	$5.0 \cdot 10^{-4}$	12.4	5.8	6.0	0.27	
HDR18	2	10–11	15	2.0	$2.3 \cdot 10^{-3}$	9.6	6.9	10.5	0.18	
HDR18	3	25–26	7	2.9	$2.0 \cdot 10^{-4}$	14.3	7.4	11.0	0.31	
HDR18	11	89–90	7	2.0	$2.8 \cdot 10^{-3}$	9.3	7.4	6.8	0.29	25.9%
HDR26	11	87–88	1	2.1	$2.3 \cdot 10^{-3}$	9.6	7.1	6.9	0.25	
HDR38	1	3–4	6	3.3	$1.0 \cdot 10^{-4}$	16.8	6.5	8.6	0.35	
HDR38	2	10–11	9	2.7	$4.0 \cdot 10^{-4}$	12.9	6.3	10.5	0.18	15.6%
VIG18	2	10–11	23	2.7	$6.0 \cdot 10^{-4}$	14.5	7.4	12.7	0.30	
VIG18	5	44–45	20	2.4	$1.4 \cdot 10^{-4}$	10.5	6.6	14.0	0.20	26.2%
VIG26	5	46–47	0	2.5	$7.0 \cdot 10^{-4}$	12.0	7.1	8.1	0.24	
VIG38	1	3–4	7	4.5	$< 10^{-4}$	22.5	7.1	11.5	0.38	
VIG38	2	10–11	4	2.8	$5.0 \cdot 10^{-4}$	12.5	6.8	11.5	0.25	
VIG38	3	26–27	17	2.2	$1.3 \cdot 10^{-4}$	10.6	6.4	5.1	0.23	22.1%

B

Trait	Linkage group	Interval	POS[a]	LOD	*P* value	*F*	F99%ile[b]	%var[c]	Δ[d]	R^2m[e]
PIL18	1	6–7	11	2.7	$4.0 \cdot 10^{-4}$	13.1	7.0	8.6	0.27	
PIL18	5	39–40	30	2.9	$2.0 \cdot 10^{-4}$	14.6	6.7	9.9	0.36	
PIL18	7	47–48	20	2.3	$1.3 \cdot 10^{-3}$	10.8	7.4	10.7	0.24	
PIL18	11	81–82	0	3.3	$2.0 \cdot 10^{-4}$	14.9	6.7	10.4	0.34	29.7%
PIL26	1	6–7	6	2.9	$3.0 \cdot 10^{-4}$	13.8	7.0	10.5	0.24	
PIL26	6	44–45	7	2.0	$3.2 \cdot 10^{-3}$	9.0	6.3	7.2	0.25	
PIL26	8	55–56	6	2.0	$2.0 \cdot 10^{-3}$	10.0	7.2	7.4	0.22	21.0%
PIL38	4	30–31	18	3.5	$1.0 \cdot 10^{-4}$	16.6	7.1	10.7	0.26	
PIL38	6	44–45	0	2.5	$8.0 \cdot 10^{-4}$	11.6	6.7	5.6	0.24	
PIL38	8	59–60	32	2.5	$4.0 \cdot 10^{-4}$	12.8	5.8	9.0	0.20	20.4%
HDR18	8	54–55	0	2.0	$2.8 \cdot 10^{-3}$	9.3	5.9	6.2	0.20	
HDR26	1	6–7	11	3.1	$1.0 \cdot 10^{-4}$	15.5	6.0	10.0	0.29	
HDR26	5	36–37	0	2.0	$3.6 \cdot 10^{-3}$	8.8	7.5	8.1	0.46	
HDR26	8	54–55	0	2.9	$3.0 \cdot 10^{-4}$	14.1	6.1	9.1	0.25	23.4%
HDR38	1	6–7	5	4.2	$< 10^{-4}$	19.9	6.6	10.7	0.26	
HDR38	2	11–12	0	2.1	$2.2 \cdot 10^{-3}$	9.6	6.1	5.1	0.20	15.6%
VIG26	1	6–7	5	2.1	$2.4 \cdot 10^{-3}$	9.5	6.9	7.6	0.20	
VIG26	2	17–18	0	2.2	$1.6 \cdot 10^{-3}$	10.4	6.0	8.4	0.34	
VIG26	5	36–37	5	2.1	$1.9 \cdot 10^{-3}$	10.1	6.6	8.1	0.23	22.0%
VIG38	1	6–7	3	3.7	$1.0 \cdot 10^{-4}$	16.7	6.5	9.5	0.24	
VIG38	2	11–12	0	2.1	$2.1 \cdot 10^{-3}$	9.7	6.8	5.1	0.18	
VIG38	6	44–45	5	2.1	$2.6 \cdot 10^{-3}$	9.3	6.2	5.4	0.21	17.7%

[a] Position of the interval from the leftmost marker
[b] 99th percentile of the *F* statistic determined by the permutation
[c] Percentage of the phenotypic variation explained by each peak
[d] The difference between the favorable QTL allele and the population mean expressed in phenotypic standard deviations
[e] Multipoint estimates of the percentage of phenotypic variation explained by the mapped QTL

of the variation respectively. No QTL was detected at 18 months. For *E. urophylla*, 2, 1 and 3 QTL accounted for 26.2% (VIG18), 8.1% (VIG26) and 22.1% (VIG38) of the variation. In both species QTL were common between two ages: for *E. grandis* VIG26-1 and VIG38-1; for *E. urophylla* VIG18-2 and VIG38-2, VIG18-5 and VIG26-5. Other QTL were specifically detected at one of the three stages.

Table 2 QTL results for *E. urophylla* (A) and for *E. grandis* (B). Shown are: RAPD marker associated with a QTL detected by two-way analysis of variance (ANOVA), *P* values associated with marker, age and marker × age effects and LOD scores obtained from the interval mapping (IM) analysis

A

Trait	Linkage group	Two-way ANOVA				IM			
		RAPD marker	Marker (*P* value)	Age (*P* value)	Marker × age (*P* value)	LOD score[a]			
						18	26	38	Interval
PIL	2	Y07_295 (10)	$7.0 \cdot 10^{-4}$	ns	0.964	2.0	2.3	–	10–11
PIL	11	X19_562 (88)	$1.4 \cdot 10^{-6}$	$1.4 \cdot 10^{-4}$	0.878	–	2.0	2.7	88–89
HDR	1	A09_586 (3)	$3.1 \cdot 10^{-3}$	$<10^{-5}$	0.036	–	–	3.3	3–4
HDR	2	T07_295 (10)	$4.6 \cdot 10^{-5}$	$<10^{-5}$	0.374	2.0	–	2.7	10–11
HDR	3	Z09_808 (25)	$4.0 \cdot 10^{-5}$	$<10^{-5}$	0.051	2.9	–	–	25–26
HDR	11	M05_722 (87)	$1.1 \cdot 10^{-6}$	$<10^{-5}$	0.529	2.0	2.1	–	87–88
VIGrt	1	A09_586 (3)	$1.8 \cdot 10^{-6}$	$<10^{-5}$	0.018	–	–	2.8	3–4
VIGrt	2	Y07_295 (10)	$4.7 \cdot 10^{-4}$	$<10^{-5}$	0.071	2.7	–	2.8	10–11
VIGrt	3	X19_709 (27)	$6.7 \cdot 10^{-5}$	$<10^{-5}$	0.063	–	–	–	–
VIGrt	5	E04_318 (46)	$4.6 \cdot 10^{-5}$	$<10^{-5}$	0.031	2.3	2.4	–	46–47, 46–47
VIGrt	10	N05_1452 (81)	$1.7 \cdot 10^{-4}$	$<10^{-5}$	0.076	–	–	2.2	80–81
VIGrt	11	M05_722 (87)	$3.7 \cdot 10^{-3}$	$<10^{-5}$	0.108	–	–	–	–

B

Trait	Linkage group	Two-way ANOVA				IM			
		RAPD marker	Marker (*P* value)	Age (*P* value)	Marker × age (*P* value)	LOD score[a]			
						18	26	38	Interval
PIL	1	X12_633 (6)	$1.2 \cdot 10^{-6}$	$4.4 \cdot 10^{-4}$	0.332	2.7	2.9	–	6–7
PIL	4	R15_625 (31)	$1.0 \cdot 10^{-4}$	$9.6 \cdot 10^{-5}$	0.240	–	–	3.5	30–31
PIL	5	U20_1358 (40)	$5.2 \cdot 10^{-5}$	$3.8 \cdot 10^{-5}$	0.076	2.9	–	–	39–40
PIL	6	N11_419 (44)	$6.8 \cdot 10^{-7}$	$4.4 \cdot 10^{-5}$	0.945	–	2.0	2.5	44–45
PIL	7	N10_2116 (48)	$3.5 \cdot 10^{-4}$	$7.1 \cdot 10^{-5}$	0.286	2.3	–	–	47–48
PIL	8	Y01_825 (55)	$8.5 \cdot 10^{-5}$	$1.2 \cdot 10^{-4}$	0.683	–	2.0	2.5	55–56, 59–60
PIL	11	B01_576 (81)	$2.5 \cdot 10^{-5}$	$2.7 \cdot 10^{-4}$	0.212	3.3	–	–	81–82
HDR	1	M12_469 (7)	$4.8 \cdot 10^{-8}$	$<10^{-5}$	0.996	–	3.1	4.2	6–7
HDR	2	R09_600 (11)	ns	$<10^{-5}$	0.003	–	–	2.1	11–12
HDR	5	N14_1588 (37)	ns	$<10^{-5}$	0.049	–	2.0	–	36–37
HDR	8	Q05_525 (54)	$2.0 \cdot 10^{-7}$	$<10^{-5}$	0.597	2.0	2.9	–	54–55
VIGrt	1	X12_633 (6)	$1.6 \cdot 10^{-5}$	$<10^{-5}$	0.028	–	–	2.5	6–7
VIGrt	2	M10_372 (15)	$8.6 \cdot 10^{-4}$	$<10^{-5}$	0.019	–	–	2.3	11–12
VIGrt	2	F04_796 (17)	$2.6 \cdot 10^{-3}$	$<10^{-5}$	0.142	–	2.3	–	17–18
VIGrt	3	Y01_494 (26)	$2.7 \cdot 10^{-3}$	$<10^{-5}$	0.010	–	–	–	–
VIGrt	5	N14_1588 (37)	ns	$<10^{-5}$	0.104	–	2.6	–	36–37

[a] For PIL and HDR, LOD scores were computed from raw data at 18, 26 and 38 months (see Table 1). For VIGrt, LOD scores were computed from incremental rates during the three periods 0–18, 18–26 and 26–38 months

Age-age phenotypic correlation

In the FS progeny, correlation coefficients for traits measured at successive stages were high and ranged from 0.81 to 0.93 ($P \leqslant 0.001$) for VIG, from 0.40 to 0.77 ($P \leqslant 0.001$) for HDR and from 0.50 to 0.65 ($P \leqslant 0.001$) for PIL. Therefore, for vigor traits fewer correlated variables than raw data were also analyzed: i.e. increments and incremental rates on a per month basis in each interval 0–18, 18–26 and 26–38.

Approximately the same QTL results were obtained (see Table 1 for the raw data and Table 2 for incremental rates). The few differences were: for *E. urophylla*, a QTL detected VIGrt38-10 (LOD = 2.2) that was not detected for VIG38-10 (LOD = 1.4), and a QTL detected for VIG38-3 (LOD = 2.2) was not detected VIGrt38-3 (LOD = 1.9) (Tables 1 and 2); for *E. grandis*, QTL detected for VIG26-1 (LOD = 2.1) and VIG38-6 (LOD = 2.1) were not detected for VIGrt26-1 (LOD = 1.4) and VIGrt38-6 (LOD = 0.4), respectively (Tables 1 and 2).

Effect of sample size on QTL detection

It must be pointed out that QTL detection was carried out on different sample sizes: 142 hybrids at 18 and 26 months compared to 201 hybrids at 38 months. In order to investigate the effect of sample sizes on marker-trait association and therefore dissociate the sampling error from any conclusion concerning the QTL stability over ages, we tested QTL detection for the traits measured at 38 months using the 142 individuals measured at 18 and 26 months. With a LOD threshold of 2.0, only 43% (6 out of 14) of the QTL detected at 38 months with 201 progeny were detected with 142 individuals at the same age (data not shown). These results confirm the theoretical and experimental results on power of QTL detection already pointed out by Darvasi et al. (1993) and Song et al. (1995), respectively.

QTL mapping in multiple years

Taking advantage of repeated measurements of the same trait at different ages, we could determine, using the two-way ANOVA analysis described in the Materials and methods, the effect on each trait when marker genotypes, year and marker × year effects were removed. Associations of marker loci with QTL linkage were considered to be significant when the F test exceeded a value necessary for a probability value less than 0.004. Results are presented in Table 2. While our annual analysis failed to detect any region consistently expressed across the three ages, this combined analysis demonstrated the existence of chromosomal segments being involved in the control of the traits across the period studied, independent of age. Almost all of the marker-trait associations detected by the two-way ANOVA had previously been detected by interval mapping (IM) at one or two ages. For PIL and HDR the same chromosomal regions were detected in *E. urophylla*, while in *E. grandis* 2 markers in LG2 and LG5 were not significant for HDR (see non-significant tests in Table 2). For VIGrt, 1 marker in LG5 was not significant in *E. grandis*, whereas new QTL were detected in LG11 of *E. urophylla* and in LG3 of *E. grandis* (Table 2).

No significant marker × year interaction ($P < 0.001$) was found for the traits studied.

Discussion

Polygenic versus oligogenic mode of inheritance for commercially important quantitative traits in *Eucalyptus*

The conventional "polygenic" model of quantitative genetics assumes that a quantitative trait exhibits continuous variation because of the combined action of environmental effects and multiple genes of small and cumulative effects. Since Sax's experiment (Sax 1923) and more recently with the development of molecular marker technologies, it has been demonstrated in plants and in animals that a trait showing a continuous distribution could be under "oligogenic"control with definable QTL potentially manipulable by the breeder. The results from our study as well as from other QTL experiments in forest trees are consistent with this latter mode of genetic control involving few major effect QTL. In *Pinus taeda*, Groover et al. (1994) detected 5 QTL for wood specific gravity that together explained 23% of the total variation. In *Eucalyptus grandis*, Grattapaglia et al. (1996) detected 5 QTL for wood specific gravity at 6.5 years, explaining 25% of the phenotypic variation. In our study, for the same *Eucalyptus* species we detected several QTL at different developmental stages. At selection age (38 months, i.e. half of the rotation age), 3 major QTL accounting for 20.4% ($R_P^2 = SS_{marker}/SS_{total}$) of the phenotypic variation (σ_P^2) of PIL were mapped in *E. grandis*. The average within-familly heritability (i.e. broad sense heritability, h^2) evaluated in the factorial design R90-11 of the breeding program (Bouvet 1995) was 0.40 for this trait. If the QTL is assumed to be tightly linked to the marker the determination coefficient could also be expressed as follows:

$$R_P^2 = \sigma_g^2/\sigma_P^2 = [\sigma_g^2/\sigma_G^2]*[\sigma_G^2/\sigma_P^2] = p^2h^2$$

where, σ_g^2 is the genetic variance explained by the QTL, σ_G^2 is the total genetic variance and p^2 was the proportion of genetic variance explained by the QTL. Thus, about $p^2 = 50\%$ of the total genetic variation for PIL38 could be ascribed to these 3 QTL. Although the loci we detected explained much of the observed variation, there may be other loci controlling variation for PIL that were not detected in this pedigree, perhaps due to lack of segregation in the cross studied. Additional loci of lesser effect may also exist but could not be detected with the sample size used (200 individuals). QTL for PIL could be especially valuable for the marker-assisted selection of wood density. Wood density is important for the production of raw material as well as for paper-making industries because it governs the quantity of the dry matter produced and is closely linked with numerous paper properties (Tissot et al. 1992).

For vigor traits, Grattapaglia et al. (1996) detected 3 QTL for diameter that accounted for 14% of the total variance. In *Populus*, Bradshaw and Stettler (1995) reported a significant QTL for second-year height that explained 25% of the phenotypic variation. In our experiment, and at 38 months, which is an excellent predictor of subsequent growth (Bouvet 1991), three QTL were detected for *E. grandis* and *E. urophylla*, accounting together for 17.7% and 22.1% of the total variation, respectively.

One QTL for stem form was also detected in the poplar experiment (Bradshaw and Stettler 1995) and explained 33.4% of the phenotypic variance. At 38 months we detected 2 QTL for *E. grandis* and *E. urophylla*, accounting together for 15.0% and 15.6% of the total variation, respectively.

Comparative QTL mapping in *Eucalyptus*

In a previous paper (Verhaegen and Plomion 1996) we identified parallel linkages of RAPD markers between our maps and those of Grattapaglia and Sederoff (1994). This alignment of linkage maps presented us with the opportunity to also compare the QTL we detected in this study to those detected for the same traits (VIG and PIL) but at different ages by Grattapaglia et al. (1996) for *E. grandis*. Indeed, QTL were detected in both studies in homologous linkage groups (Table 3). This encouraging result makes it possible to detect QTL alleles in a large range of genetic backgrounds, which is a prerequisite to using molecular markers for breeding purposes.

Clustering of QTL affecting different traits

Several QTL for VIG and HDR were detected in the same genomic regions (for *E. urophylla* in LG1 and LG2 at 38 months; for *E. grandis* in LG1, LG2 at 38 months and in LG5 at 26 months). This cosegregation was not surprising because of the high correlation coefficient between both traits ($\rho = 0.68$–0.79; $P \leqslant 0.001$). HDR was computed from height and circumference and is a surrogate for stem taper. It was clearly associated with straightness of the trunk as well as with yield. Although a pleiotropic gene action might be the most likely explanation for these frequent QTL colocalizations, the present data were insufficient to distinguish between pleiotropic effects of a single gene and the independent effects of tightly linked loci controlling several traits.

Table 3 Comparative QTL mapping in *E. grandis* between results reported in this study (V et al.) and those by Grattapaglia et al. (1996; G et al.). Traits measured were wood density: pilodyn pin penetration depth (PIL) or wood specific gravity (WSG), and vigor: total height or circumference at breast height (VIG). Traits were measured at ages that varied from 18 to 78 months

Homologous linkage groups		Detected QTL	
V et al.	G et al.	V et al.	G et al.
LG1	LG2	PIL18, PIL26	WSG78
		VIG26, VIG38	VIG78
LG8	LG5	PIL26, PIL38	WSG78
		VIG18, VIG26	VIG78
LG10	LG11/13	No	No

An other interesting feature was the overlapping between QTL for PIL and VIG or HDR in LG2 and LG11 in *E. urophylla* and in LG1, LG6 and LG8 in *E. grandis*. This result was in agreement with the significant correlation between these traits (an average of $\rho = 0.5$; $P \leqslant 0.001$). This positive correlation between pilodyn pin penetration depth and growth may be associated to the negative correlation between wood density and vigor. A negative relationship between density and growth has already been reported (Malan 1988, Malan 1991), and it seems that there is an apparent contradiction between selecting for growth (mostly diameter growth) and selecting for high wood density in these species. For 3 QTL colocalizations between PIL and VIG, the allele that increased growth also increased the penetration of the pilodyn pin: for *E. grandis* VIG26-1 and PIL26-1, VIG38-6 and PIL38-6, and for *E. urophylla* VIG18-2 and PIL18-2. If a tight linkage of genes is causing this negative relationship it might be possible to break the association by further recombination in these segments. Theoretically, favorable recombinants could be detected by molecular markers that reside in these chromosomal regions. The possibility also exists that the same genetic factors control both characters (pleiotropy). While, it is unlikely that selection for both traits will be efficient based on these QTL, it could still be possible to improve these two traits together, by selection for the RAPD makers associated with independent QTL of PIL and VIG.

Breaking unfavorable correlations between quantitative characters of interest and subsequently creating genotypes with a desired trait configuration has been reported as one of the possible outcomes of QTL mapping experiments. This is the case for traits related to yield and early maturity in many crop plants (Lindhout et al. 1994; Zhikang et al. 1995), fresh yield and solubesolids content of the fruit (Brix) in tomato (Tanksley and Hewitt 1988; Eshed and Zamir 1994; Paterson et al. 1991), yield and malting quality in barley (Hayes et al. 1993). Our results for PIL and VIG showed that it should be possible to select for fast-growing eucalyptus trees with dense wood.

Marker-assisted selection (MAS) of hybrid clones

Stability of QTL expression until selection age

Trees are long-lived organisms that undergo maturation processes. Progressive changes in vegetative characteristics occur at the morphological, anatomical and physiological level. In addition to these internal modifications, trees are faced with a wide spectrum of climatic conditions during their lifetime. Modification in QTL expression over diverse environments has already been reported in crop plants (Paterson et al. 1991; Hayes et al. 1993) as well as in fruit trees (Asins et al. 1994). In forest trees, the instability of QTL expression over age

Table 4 Proportion of QTLs expressed at one or two ages for the studied traits

Months	18	26	38
Shared QTL	26.3%	31.6%	10.5%
Specific QTL	10.5%	5.3%	15.8%

has already been reported in poplar for basal area (Bradshaw and Stettler 1995) and in pine for juvenile growth (Plomion et al. 1996b). On the basis of our annual analysis, a partial overlap of QTL was found during the period studied, with 68.4% of the QTL being detected at two ages and 31.6% being age-specific (Table 4). No QTL were consistently expressed at 18, 26 and 38 months. From a statistical standpoint, the relatively heterogeneous behavior of the QTL over all these ages could be caused by the different power of QTL detection associated with measurements at 18 and 26 months on the one hand (140 individuals measured) and at 38 months on the other hand (201 individuals measured). Indeed, 8 of the 14 QTL detected at 38 months would not have been detected by analyzing a restricted sample size of 140 individuals. It is worth noting that half of these QTL were age-specific. Thus, our conclusion would not have been changed by analyzing a constant sample size of 140 trees at 18, 26 and 38 months. If we assume that the same set of structural genes is involved in the traits studied, this result might show that the same set of regulatory genes differentially control the expression of the traits across ages or, alternatively, that different sets of regulatory factors are involved during different periods of time. However, we can not distinguish between these hypotheses.

The two-way ANOVA was powerful and made it possible to investigate the marker effect independently from year and marker × year interaction effects. Results showed that the genomic regions detected by IM in the annual analysis were also involved in the control of the traits across the period studied, except for a few cases presented in the Results section. It is possible that strong effects of QTL within a single year may persist when the analysis is conducted over several years. Despite the absence of a QTL × year interaction, there was a variable expression of QTL from year to year: no QTL was consistently expressed at 18, 26 and 38 months. In the *Eucalyptus* breeding program, the actual selection age of hybrid individuals is 3 years (Bouvet 1991). In order to define the optimal age for early selection for vigor traits of *Eucalyptus* clones, Bouvet and Vigneron (1995) have recently studied the age trends in broad-sense heritabilities on an individual basis. Their results indicated that the early selection of individual trees to be vegetatively propagated for clonal variety deployment might be practical after the first year of assessment. Our results favor an early screening based on marker information.

Selection of hybrid genotypes for vegetative propagation

The RRS scheme of eucalyptus aimed at producing clonal hybrid varieties for reforestation in the Congo. Clonal selection of eucalyptus is a time-consuming process that involves within-family selection followed by two successive clonal tests (Delwaulle 1988). In a first-generation clonal test (CT1), 25–50 clones per selected hybrids are planted in a non-replicated design. It is basically used to screen for the best genotypes with a moderate selection intensity (15–30%). In a second-generation clonal test (CT2), genotypes that previously presented good performances are planted and compared in a replicated design (16 clones with five replications). Approximately 6% of them will be vegetatively propagated for reforestation. At the present time, CT1 and CT2 last approximately 6 years each. In addition, Bouvet (1995) recently showed that while early selection of *Eucalyptus* hybrid clones at the nursery stage would allow the elimination of the worst genotypes, it would not be without the risk of losing good performers. In that context, the introduction of molecular markers in the selection process should improve the efficiency of hybrid selection within virtually any FS family (Grattapaglia and Sederoff 1994; Plomion et al. 1996a). On the one hand, the accuracy of the within-family selection, especially for low heritability traits, could be increased by choosing hybrid offsprings that carry favorable quantitative trait alleles (QTA) and have a superior phenotype. On the other hand, a higher within-family selection intensity could be applied, even at a younger age (see below), reducing the number of individual clones to be tested in the field. This pre-screening could be followed by one single clonal test instead of two. This would ultimately lead to increased genetic gain per unit of time by decreasing the generation interval. This hypothesis needs to be tested and compared to classical clonal selection. Hybrid trees selected on the basis of markers could be followed until clonal plantation at rotation age to verify the efficiency of the proposed MAS strategy.

Sample size needed for within-family MAS

The probability P of recovering at least one individual carrying N favorable QTAs, depends on the size of the fragments flanking the N QTL and can be expressed as (Plomion et al. 1996a):

$$P = 0.5*(1 - r_1)*(1 - r_2)^N$$

where r_1 and r_2 are the map distances in centiMorgans between adjacent markers bracketing the QTL peak

(the extreme markers bracketing the 1.0 LOD support interval could be used to define this confidence interval). For example, for confidence intervals of 20 cM surrounding the most probable location of the QTL (e.g. $r_1 = r_2 = 10$ cM), P takes values of 0.405, 0.164, 0.066, 0.027, 0.01 and 0.00012 for N = 1, 2, 3, 4, 5 and 10 QTL, respectively. Thus, a sample size of 200 individuals would be required to find a transgressive individual carrying N = 5 favorable QTAs. Strauss et al. (1992) have also shown that 200 individuals are needed to detect half of the additive variance with a Type-I error of 1% when the heritability (*sensus stricto*) of the trait is 0.5 and 5 QTL control the trait. Because such high heritability is rarely obtained in forest trees (Cornelius 1993), 200 must be viewed as a minimum size. This size could, however, be reduced with the use of clonal replicates (Bradshaw and Foster 1992).

Eucalyptus breeders are interested in the improvement of vigor and form of the stem as well as wood density. Our results showed that simultaneous improvement of multiple traits using molecular markers will only be feasible with a high number of progeny. However, huge sample sizes are usually not available in most operational forest tree breeding programs. Therefore, even if clonal propagation should allow the amplification of highly valuable genotypes identified by MAS, we can reasonably predict that within-family MAS of hybrid trees for vegetative propagation will be faced with choices of priorities of one trait over another as is already the case in conventional breeding.

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