Detection of quantitative trait loci in *Pinus sylvestris* L. across years

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Summary

In an earlier study, we reported the characterisation of quantitative trait loci (QTLs) for economically important traits related to wood production for a specific year of measurement. However, validating the detected QTLs across years is important for any strategy for marker-assisted selection (MAS). Therefore, we evaluated the consistency of the QTLs across four years in 9 to 12 year-old trees. Data related to tree height were analysed in two ways. The total height was used to characterise QTLs likely to be useful in MAS, and the increment values to evaluate growth regulation. Among the 11 QTLs detected, 4 were found in both the 1996 and 1997 evaluations. QTL numbers and locations related to total height were found to be highly stable. However, the QTL effects progressively changed in successive years, following either an increasing, decreasing or curvilinear trend. Depending on their specific trends, some QTLs may not be expressed at all later in maturity, or they may be expressed at a higher level. This will have direct consequences on the applicability of molecular markers in early screening programs, since a study at the mature stage may also be required. More variation in QTL number and effect was observed relating to height increment than to total height, indicating significant differences in gene expression during the growth periods involved.

Introduction

Sweden has a long tradition of forest tree breeding, focused on the establishment of seed orchards until 1981. A new breeding program for *Pinus sylvestris* was then proposed and further developed in the early 1990s (Wilhelmsson & Andersson, 1993), following a recurrent selection scheme, combining both long- and short-term goals via the establishment of multiple populations and the development of elite lines.

Molecular markers enable the genomic location to be determined for loci controlling the expression of quantitative traits (i.e. quantitative trait loci, or QTLs). Contradictory statements have been made about the use of marker related methods in selection (marker-assisted selection or MAS) of forest tree species (e.g. O'Malley & McKeand, 1994; Plomion et al., 1996b; Strauss et al., 1992). As pointed out by Strauss et al.

(1992), before markers are integrated into such programmes it is essential to verify several key features, especially that the QTLs are stable across appropriate ranges of environmental factors and genetic backgrounds. Moreover, a major goal might be to use the markers as tools for early screening, an important consideration when dealing with long cycle species (Neale & Williams, 1991). If MAS can shorten evaluation cycles, it may have much greater impact on forest breeding than on annual crops.

Usually, very weak correlations have been reported in *Pinaceae* between height values at the juvenile and mature stages (Lambeth, 1980; Kremer, 1992), which hinders early selection. In seedlings of *P. pinaster*, Plomion et al. (1996a) also observed a variation in expression of QTLs related to height between two periods of growth. If this tendency is confirmed over

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longer periods, it will have important consequences for the use of markers in early selection stages.

In an earlier study, we characterised QTLs for economically important traits related to wood production for a specific year (Lerceteau et al., 2000). Further measurements have now been carried out to evaluate the consistency of the QTL location and effect across four years corresponding to 9 to 12 year-old trees. The new data presented in this paper were used in two ways: the total height data to provide information on which QTLs would be potentially useful in MAS programs and the increment data to obtain insight into the genetic control of growth regulation.

Material and methods

Mapping population

For genetic map development and QTL detection, 94 trees of a single full-sib family were used. Both parental trees (accession AC3065 used as female parent, and accession Y3088 used as male parent) are plus-trees originating in northern Sweden (latitude 65°08′ and latitude 64°09′ respectively), and both are currently used in Swedish breeding programs. The progeny, available from the Forestry Research Institute of Sweden, were planted at one location, at latitude 64°02′, in spring 1988 when they were one-year old seedlings.

Phenotypic measurements

Total tree height from the ground level to the terminal bud was measured in autumn 1999 at the whorl corresponding to bud 1996 (H96), 1997 (H97), 1998 (H98) and 1999 (H99). Because height values were cumulative, the following height increments were computed from these data: H96/H97inc = H97-H96; H97/H98inc = H98-H97; H99/H98inc = H99-H98. Trunk diameter at breast height, i.e. 1.3 m from ground level was measured in spring 1997, corresponding to trunk diameter at the end of the 1996 growing season (DBH96), and in autumn 1999 (DBH99). Measurements of trunk diameter at 0.5 m from the ground were also taken at the same time (D0.5/96 and D0.5/99). Branch diameter was measured in spring 1997 (BRCD96) of the 'average' branch at the fourth branch level from the terminal bud and in autumn 1999 (BRCD99) at the seventh branch level (i.e. the same whorl and branch as in 1997). Branch angle values (BRCA96, BRCA99) of the 'average' branches were

Table 1. Total variance explained by the QTLs

Traits	Number of QTLs	R^2t^a (%)
H96	3	32.2
H97	3	33.6
H98	3	33.9
H99	3	34.3
H96/97inc	1	15.3
H97/98inc	1	15.4
H98/99inc	1	14.9
BRCD99	2	29.5
BRCA96	1	16.1
DBH96	1	15.0
DBH99	3	27.3
D0.5/96	1	14.0
D0.5/99	1	11.9

[a] Total phenotypic variance explained by QTLs.

also measured. For branch angle, and both branch and trunk diameter, no increment was calculated since the measurements were carried out only twice, in spring 1997 and autumn 1999.

Marker-trait associations

DNA extraction and the AFLP detection procedure were performed as described by Lerceteau & Szmidt (1999) using the AFLP™ system from Gibco BRL, Life Technologies. Genetic linkage maps (maternal and paternal) were developed following a double pseudo-test cross strategy, as reported elsewhere (Lerceteau et al., 2000).

QTL analysis was performed on each parental map with MAPMAKER/QTL 2.0 software (Paterson et al., 1988), setting a LOD score threshold of 2.0 to accept the presence of a linked QTL in the interval.

Results

Normality of the distributions

The normality of the distributions of the quantitative traits was evaluated using the Kolmogorov-Smirnov test. Slight or significant departure from normality was observed for most of the detected traits. However, after transformation (square, square root, inverse or logarithmic), most of them followed a normal distribution, with the exception of BRCA99, BRCD96 and H97/98inc.

Table 2. Percentage of phenotypic variation explained by each QTL for height control for the female parent, AC3065. No QTL was found for the male parent, Y3088. In parentheses: Peak LOD value. In italics: QTLs with a LOD value under the 2.0 threshold of significance. For the interval see in Lerceteau et al., 2000.

QTL	Interval	Linkage group	H96	H97	H98	H99	H96/97 inc	H97/98 inc	H98/99 inc
HF3	acg/ctc8+//acg/cag13+	F3	18.2	16.6	15.5	13.1	*10.4*	*9.0*	*3.4*
			(3.25)	(2.97)	(2.81)	(2.64)	*(1.67)*	*(1.53)*	*(0.66)*
HF7	acg/cac13-//acg/ctg21-	F7	11.5	10.8	10.4	12.0	*4.5*	*2.5*	14.9
			(2.43)	(2.27)	(2.16)	(2.54)	*(0.90)*	*(0.49)*	(3.19)
HF12	agc/ctc8-//acgc/cat18+	F12	9.6	11.9	14.8	14.4	15.3	15.4	*3.1*
			(2.01)	(2.52)	(3.18)	(3.06)	(3.28)	(3.35)	*(0.61)*
R^2m[a]			32.2	33.6	33.9	34.3	15.3	15.4	14.9

[a] Percentage of phenotypic variation explained by all the QTLs (multiple regression analysis).

QTL analysis

In total, for all traits, in both male and female plants and considering all years, 11 different QTLs were detected. Four QTLs were associated with DBH, three with height, two with branch diameter, one with D0.5 and one with branch angle. For the traits measured in spring 1997 (representing data values from 1996) 6 QTLs were observed, compared to 9 in 1999. Four out of 11 QTLs (36.4%) were found in both the 1996 and 1999 evaluations. Thus, taking two sets of samples, three years apart, allowed the detection of 7 additional QTLs. The percentage of total variance explained by all the QTLs detected for specific traits in the parent trees is given in table 1. The proportion of the variance explained by all the detected QTLs controlling specific traits ranged from 11.9 (D0.5/99) to 34.3% (H99) and on average equalled 22.6 ($\pm$ 9.1). Since the female and male maps had no common markers, it was not possible to ensure that the same QTLs were not detected both on the male and female sides. If some common QTLs have been observed, the proportion of the effects explained by the QTLs might be overestimated since they would have been counted twice.

QTLs associated with height and height increment

Analysis of the total tree height collected over the four sampling years identified three QTLs on the female map (*HF3*, *HF7*, and *HF12*) (Table 2). The LOD score values suggested that the expression of *HF3* declined across the years, whereas a general rising trend was observed for *HF12*, but with a slight dip in 1999. Like *HF3, HF7* was generally expressed increasingly weakly, but the LOD value increased again in 1999. The percentages of total phenotypic variation explained by the four QTLs together were nearly equal (varying from 32.2 to 34.6%) in 1997, 1998 and 1999.

Only two out of three QTLs (*HF7* and *HF12*) were detected in an analysis of the height-increment data sets. The patterns of expression were highly irregular and none of them was found for all of the incremental years. *HF12* was associated with the growth periods of 1997 and 1998. Its expression level was constant for these two periods, but then decreased considerably to below the level of significance. *HF7* was associated with the growth period of 1999.

On the male map, no QTL was detected both for total tree height and for height increment.

QTLs associated with other traits

Six QTLs related to branch and trunk characters were localised on the female map (Table 3A), the number per trait ranging from one to two. On the male map, 3 QTLs were detected (Table 3B). Comparison of QTLs observed for the same trait measured in different years showed that in only one case (D0.5/96 and D0.5/99 on linkage group F7) a QTL was common to both observations. In some cases and for both maps (e.g. DBH96 and DBH99 on linkage group F7 or BRCD96 and BRCD99 on linkage group M1), common QTLs were detected, but for one of the two occasions with a LOD value slightly below the level of significance. Co-locations of QTLs for different traits were also observed. For example, the QTL on the linkage group F14 seemed to influence branch and trunk diameter in 1999.

Table 3. QTL results for the female, AC3065 (A), and the male, Y3088 (B), parents In italics: QTLs with a LOD value under the 2.0 threshold of significance. For the interval see in Lerceteau et al., 2000

A – AC3065

Trait	Linkage group	Position on the map	LOD	% var[a]	R^2m[b]
BRCD96	–	–	–	–	–
BRCD99	F14	acg/cta38+//acg/ctc4-	2.51	13.1	13.1
BRCA96	F5	agc/ctg19-//agc/ctc21-	3.00	16.1	16.1
BRCA99	*F5*	*agc/ctg19-//agc/ctc21-*	*1.83*	*11.7*	11.7
DBH96	*F7*	*acg/cac13-//acg/ctg21-*	*1.92*	*9.3*	
DBH99	F7	acg/cac13-//acg/ctg21-	3.15	14.7	
	F14	acg/cta38+//acg/ctc4-	2.31	11.3	21.0
D0.5/96	F7	acg/cac13-//acg/ctg21-	3.02	14.0	14.0
D0.5/99	F7	acg/cac13-//acg/ctg21-	2.49	11.9	11.9

B – Y3088

Trait	Linkage group	Position on the map	LOD	% var[a]	R^2m[b]
BRCD96	*M1*	*acg/cag9+//agc/ctc3-*	*1.91*	*10.8*	
BRCD99	M1	acg/ctc22+//agc/ctc3-	3.12	14.9	14.9
DBH96	M9	agc/ctc1+//agc/ctg18-	2.42	15.0	15.0
DBH99	*M9*	*agc/ctc1+//agc/ctg18-*	*1.90*	*12.1*	
	M10	acg/cac9-//agc/ctc12-	2.02	10.8	10.8

[a] Percentage of phenotypic variation explained by each peak.
[b] Percentage of phenotypic variation explained by all the QTLs (multiple regression analysis).

Discussion

In this paper we compare QTLs related to *Pinus* growth characters detected in samples from different years. Data related to samples taken solely in spring 1997 have been discussed elsewhere (Lerceteau et al., 2000).

Globally, few QTLs per trait were detected. Higher numbers of QTLs were found for total height and breast height diameter than for the other characters evaluated. According to Hannrup et al. (2000) these traits are less strongly influenced by environment than branch-related traits, branch angle excluded, so the probability of identifying QTLs linked to them is higher. As reported by Kaya et al. (1999) in a study of *Pinus taeda*, the apparent presence of a small number of QTLs with strong effects is not in total accordance with the infinitesimal model (which assumes that quantitative traits are influenced by many genes with small and additive effects). However, the analysis of a small number of individuals can lead to overestimation of the QTL effect (Beavis, 1994). Even if the number of mapped markers is less important for QTL analysis

than the number of individuals (Darvasi et al., 1993), the observed percentages of genome coverage of our maps (in Lerceteau et al., 2000) suggest that some parts of the genome are not covered. Therefore, both the possible over-estimation of the effects of QTLs, and our inability to detect non-polymorphic and minor QTLs or QTLs located in unmapped regions, lower the estimation of the number of genes involved in the control of a trait.

Both total height and incremental data were used in the height analysis. For marker-assisted selection purposes, the total height values will be more useful than the height-increment analysis. It represents the total height reached, cumulated over all the growing seasons, and thus provides a global perspective of great interest to breeders. On the other hand, height-increment data give information about the rate of growth and the stability of QTL expression across the years, and thus insight into the QTLs expressed, and their strength of expression, during specific growth periods. These data are important for understanding the genetic basis of growth regulation.

The analysis of total height showed that both the number and the location of QTLs detected in any one year was conserved across the years. Thus, from 1996 to 1999 detection of the QTLs was mostly independent of the year of measurement and, to a degree, results from each year were predictive for results from the other years (and similar information would have been obtained using data from any one of the sampling periods). However, few years were analysed, representing a small portion of the tree life cycle. In *Cryptomeria japonica*, QTLs for height detected in both four- and five- year old trees were not confirmed at the later age of 14 (Yoshimaru et al., 1998). A similar pattern may be observed in our investigation. The trees are older (12 year-old) but they are still at the juvenile stage and the presence of the same QTLs at the mature stage has yet to be verified. Moreover, the LOD values of the QTLs changed over the years, variously following increasing, decreasing or curvilinear trends. The significance of the trends can not be precisely assessed without evaluating data from further years. Then, if the trends are genuine, some QTLs may become non-significant at mature stages and thus lose their predictive value (see e.g. *HF3*), or their level of expression may become stronger, thus retaining (or increasing) their usefulness. Based on the estimation of Lambeth (1980) related the age-age correlation for height in *Pinaceae* and considering the rotation time for *P. sylvestris* in northern Sweden to be 80 to 100 years, the correlation between heights of 12 and fully mature trees will vary between 0.3 and 0.5. These values are much lower than the observed correlations between the four sampling years (from 0.95 (p <0.0001) for HT96/HT99 to 0.99 (p <0.0001) for HT98/HT99). Therefore, as previously reported by Conner et al. (1998), based on results from a study of apple trees, measuring mature trees might be the best method for detecting QTLs that will be most useful in selecting for tree vigour. Height evaluations are particularly difficult for pine when trees are tall, but it might be essential for determining the earliest possible ages at which QTLs can be detected that will remain constant up to maturity. Even better would be to find early QTL expression for mature volume. Volume is the most common goal trait in selection and is indirectly selected for by height at young age. The utility of markers as a tool for early screening would greatly benefit from such a determination.

The relative stability observed, at least in the number of QTLs in our study, contrasts markedly with the results reported by Emebiri et al. (1998) in *Pinus radiata*. Based on total height obtained from 5 months, 1, 2 and 3 year-old trees, these authors detected 10 QTLs associated with height, among which none was expressed at all of the four ages. Forty-five percent of them had a limited stability, being present at two subsequent stages. The reason for the difference in QTL stability between the two studies may be related to differences in the developmental stage of the trees, very early for the *P. radiata* and more advanced in our study. This suggests that QTL expression may be more stable after a certain age, but more data are required to confirm this hypothesis.

The fairly high stability we found across the years (1996 and 1999) of the QTLs associated with height contrasted with the significantly greater variation observed for branch- and other stem- related traits. QTLs common to both years were found for branch and stem characters, but often for one occasion the LOD value was below the significance level. Moreover one additional QTL was detected in just one year. In *Pinus pinaster*, diameter is considered to be more sensitive to environmental conditions than height (von Euler et al., 1992), which may explain our results.

Different QTLs related to height-increment were expressed at different levels during specific growth periods. The QTLs detected in the height-increment data analysis appeared to vary dramatically in their level of expression across the growth seasons. Similar results have been reported in studies of other *Pinus* species, including *P. pinaster* (Plomion et al., 1996a) and *P. taeda* (Kaya et al., 1999) and in other genera, e.g. *Eucalyptus* (Verhaegen et al., 1997) and *Malus × domestica* (Conner et al., 1998). Plomion et al. (1996a) suggested that different regulatory genes or differential expression levels of the same set of regulatory genes may be involved at different stages of maturation. Emebiri et al. (1998) also reported a general pattern of QTL activation and repression throughout the study period, suggesting that genes were being switched on and off. Based on their total height data set, they described three trends of influence displayed by different QTLs. A question that remains unresolved is whether there are one or several genuine trends of expression for particular genes over the lifetime of trees, or if their expression is limited to specific temporal periods defined by environmental cues.

The large changes in QTL expression levels observed in increment values contrasted with the relative stability of QTLs for total height. It might be that the years before investigations have been the most important for growth so far, in comparison to years 1996 to

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1999. As reported by Costa & Durel (1996), genomic expression could fluctuate widely when differences between annual climatic conditions are strong. On the other hand, cumulated height increments may allow more reliable expression of the genetic potential and reduce environmental effects. Thus, QTLs detected using cumulated height data may also be more reliable than those detected in short-term incremental analyses.

Acknowledgements

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