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Mapping of quantitative trait loci controlling adaptive traits in coastal Douglas-fir. I. Timing of vegetative bud flush

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Abstract Thirty three unique quantitative trait loci (QTLs) affecting the timing of spring bud flush have been identified in an intraspecific mapping population of coastal Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco var. *menziesii*]. Both terminal and lateral bud flush were measured over a 4-year period on clonal replicates at two test sites, allowing for the repeated estimation of QTLs. QTLs were detected on 12 linkage groups and, in general, each explained a small proportion of the total phenotypic variance and were additive in effect. Several QTLs influence the timing of bud flush over multiple years, supporting earlier evidence that the timing of bud flush through developmental stages is under moderate to strong genetic control by the same suite of genes through developmental stages. However, only a few QTLs controlling the timing of bud flush were detected at both test sites, suggesting that geographic location plays a major role in the phenology of spring growth. A small number of QTLs with year and site interactions were also estimated.

Keywords QTL mapping · RFLP · Bud phenology · Genotype×environment interaction

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Introduction

Adaptive traits in Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco var. *menziesii*] are of practical interest to tree breeders and gene-resource managers in the Pacific Northwest. Douglas-fir populations are adapted to diverse environments throughout its range and express a large amount of genetic variation in adaptive traits, such as frost-hardiness (Rehfeldt 1979; Aitken and Adams 1996, 1997), drought-tolerance (Larsen 1981; White 1987), and bud phenology (Christophe and Birot 1979; Campbell 1986, 1987; Joly et al. 1989; El-Kassaby and Park 1993; Li and Adams 1993). The ability of long-lived organisms, such as conifers, to adjust to various environmental conditions is paramount to fitness, especially since reproduction does not occur for many years.

The timing of spring bud flush in Douglas-fir is an important adaptive trait (Campbell and Sorensen 1978; White et al. 1979; Loopstra and Adams 1989; Li and Adams 1993). This phenological trait plays a critical role in initiating the annual growth cycle early in the spring while soil moisture is high, yet late enough to avoid spring frost damage. Frost damage to new shoot tissue can retard growth, cause stem-defect and, in severe cases, kill young trees (Campbell 1986; Li and Adams 1993; Schermann et al. 1997). In one study, sapling height growth, spring bud flush, and spring cold-hardiness were found to be strongly associated at one site, yet weakly associated at another (Aitken and Adams 1995). The growing season of Douglas-fir varies in relation to its climatic and geographical environment, with bud flush occurring earlier in lower elevations and at lower latitudes (Campbell and Sorensen 1978; Rehfeldt 1989). Environmental factors, such as photoperiodicity, temperature and winter chilling, induce cell cycling and the elongation of the meristematic tissue in the spring (Campbell and Sugano 1975; Campbell and Sorensen 1978; Steiner 1979; Bigras and D'Aoust 1993; Hanninen 1995). The manner in which these environmental cues interact with genetic components that control the timing of bud flush in conifers has not been determined.

The timing of bud flush is under moderate to strong genetic control, with individual tree or family heritabilities ranging between 0.44 and 0.95 (Christophe and Birot 1979; Rehfeldt 1983; Li and Adams 1993; Aitken and Adams 1997). Li and Adams (1993) reported high heritabilities and phenotypic stability among families across test environments for seedlings and pole-sized trees and conjectured that bud flush phenology in these stages of development is controlled by the same suite of genes. However, the number of genes involved in the induction and expression of bud flush remains unknown, and the interaction between genetic factors and environmental cues regulating its expression is poorly understood.

In this study, our goal is to begin to identify the genes that control the timing of bud flush by mapping the individual genetic loci contributing to this polygenic trait. Quantitative trait loci (QTLs) controlling the timing of bud flush were estimated and mapped using a restriction fragment length polymorphism (RFLP) linkage map that was constructed from a three-generation outbred pedigree (Jermstad et al. 1998). The progeny were cloned by vegetative propagation and planted at two test sites. Spring bud flush was scored for 3 years at the Oregon site and for 4 years at the Washington site. QTLs were estimated by using the multiple marker interval mapping method reported in Knott et al. (1997). We have detected 33 unique QTLs controlling spring bud flush in Douglas-fir, each explaining only a small proportion of the total phenotypic variance.

Table 1 Description of spring bud flush trait, year of measurement, and name of the trait analyzed

Trait description	Year	Trait name
Washington Site (n[a]=190)		
Lateral bud flush	1995	*wlat*95
Lateral bud flush	1996	*wlat*96
Lateral bud flush	1997	*wlat*97
Terminal bud flush	1995	*wter*95
Terminal bud flush	1996	*wter*96
Terminal bud flush	1997	*wter*97
Bud flush	1998	*wflu*98
Oregon Site (n=78)		
Lateral bud flush	1995	*olat*95
Lateral bud flush	1996	*olat*96
Terminal bud flush	1995	*oter*95
Terminal bud flush	1996	*oter*96
Bud flush	1998	*oflu*98
QTL×year and QTL×site interaction analyses		
Washington year interaction[b]	1995, 1996	*year*WA
Oregon year interaction[c]	1995, 1996	*year*OR
Site interaction[d]	1995	*site*95
Site interaction[e]	1996	*site*96
Site interaction[f]	1998	*site*98

[a] Number of clones in sample
[b] *wter*95 and *wter*96 data included in analysis; *n*=190
[c] *oter*95 and *oter*96 data included in analysis; *n*=78
[d] *oter*95 and w*ter*95 data included in analysis; *n*=78
[e] *oter*96 and w*ter*96 data included in analysis; *n*=78
[f] *oflu*98 and w*flu*98 data included in analysis; *n*=78

Materials and methods

Plant materials

A three-generation outbred pedigree was constructed for the purpose of mapping QTLs that control the timing of bud flush. Grandparent pairs were selected based on the timing of spring bud flush of grafted ramets in operational seed orchards. Individuals representing the early and late ends of the bud flush spectrum were crossed in each of two grandparent pairs, producing two F_1 families. Two F_1 individuals, one from each family, were mated to each other to produce F_2 progeny. Forty eight of the progeny were selected and grown for collecting needle tissue for DNA isolation (Jermstad et al. 1998). In the spring of 1993, vegetative cuttings were taken from the remaining seedlings, rooted under cover (Ritchie 1993) and planted at Weyerhaeuser Company nursery bed sites in Mima, Washington, and Aurora, Oregon, in August 1993.

In April 1995, the rooted cuttings were lifted and transferred to permanent test sites in Twin Harbors, Washington, and Turner, Oregon. The site in Washington is located on a mountainous north-facing slope at an elevation of 122 m that had previously been logged, leaving stumps and much microenvironmental variation. In contrast, the Oregon site, located in the Willamette Valley, is a uniform flat field at an elevation of 88 m, with milder temperatures. The mean annual rainfall for the Washington and Oregon sites is 216 and 99 cm, respectively. Although the longitude is roughly the same for both sites, the Washington site is located approximately 241 kilometers north of the Oregon site. An incomplete randomized block design was used with four blocks per site, and clones were planted in 3-tree row plots. There were an insufficient number of clones to make a complete test at both sites. The Washington site was planted first and the remaining ramets from clones were used to establish the Oregon site. In the fall of 1997, there were 224 clones at the Washington site with an average of eight ramets per clone and 78 clones at the Oregon site with an average of ten ramets per clone.

Phenotypic measurements

The timing of vegetative bud flush was recorded at each test site for several years. At the Oregon site, measurements on terminal and lateral bud flush were made in the spring of 1995 and 1996 (Table 1). Likewise, at the Washington site, measurements were made on terminal and lateral bud flush in the spring of 1995, 1996 and 1997. Buds were considered flushed when green needles had visibly shed bud scales. Terminal buds were scored as either flushed (1) or not flushed (0) on a given day. Lateral bud flush scores were assigned on a whole-crown basis: <50% of lateral buds flushed (0) or ≥50% of lateral buds flushed (1). In 1998, terminal bud flush was measured at both sites, using a more-refined scoring system that recorded the stage of bud flush: 1= bud tight and dark; 2=bud closed, swollen and white; 3=buds open slightly, needles less than 1-cm long; 4=buds open, needles 1–5-cm long; 5=buds open, needles more than 5-cm long. All measurements were made on a single Julian date in which an intermediate percentage of the clones had flushed. This simple method of measurement detects the best differentiation among families for bud flush when approximately 50% of the population is flushed. Clonal means were calculated at each test site for 1995, 1996, 1997 and 1998.

Test-site statistics

Test-site means, coefficients of variation among clones, and the measure of skewness in the frequency distributions of clones were calculated for each trait by using PROC UNIVARIATE by Systems Analysis Software, Inc. (SAS 1989–1996). The terminal bud

Table 2 Test site means, coefficients of variation (CV) among clones, and measures of skewness in the frequency distribution for bud flush traits among clones as calculated by PROC UNIVARIATE (SAS 1989–1996) (see Table 1 for description of trait names)

Traits	Washington Site			Traits	Oregon Site		
	Mean	CV (%)	Skewness		Mean	CV (%)	Skewness
*wter*95	0.58	54	–0.24	*oter*95	0.60	50	–0.35
*wlat*95	0.69	42	–0.70	*olat*95	0.78	31	–0.88
*wter*96	0.41	84	0.30	*oter*96	0.70	33	–0.84
*wlat*96	0.80	32	–1.38	*olat*96	0.86	42	–1.79
*wter*97	0.55	65	–0.34	*oter*97	–	–	–
*wlat*97	0.75	42	–1.07	*olat*97	–	–	–
*wflu*98	3.19	18	–0.33	*oflu*98	3.64	21	–0.14

Table 3 Estimated clonal correlations among bud flush traits (see Table 1 for description of trait names)

Trait	*oter*95	*olat*95	*oter*96	*olat*96	*oflu*98	*wter*95	*wlat*95	*wter*96	*wlat*96	*wter*97	*wlat*97	*wflu*98
*oter*95	–	0.77	0.61	0.38	0.50	0.58	0.50	0.53	0.45	0.56	0.59	0.49
*olat*95		–	0.60	0.33	0.49	0.49	0.53	0.47	0.49	0.44	0.62	0.48
*oter*96			–	0.56	0.47	0.43	0.47	0.49	0.51	0.45	0.57	0.50
*olat*96				–	0.47	0.36	0.40	0.39	0.51	0.31	0.43	0.42
*oflu*98					–	0.49	0.50	0.71	0.70	0.76	0.71	0.78
*wter*95						–	0.76	0.46	0.40	0.47	0.42	0.47
*wlat*95							–	0.47	0.42	0.47	0.46	0.43
*wter*96								–	0.64	0.53	0.55	0.62
*wlat*96									–	0.49	0.61	0.70
*wter*97										–	0.75	0.61
*wlat*97											–	0.67
*wflu*98												–

flush scores for 1995, 1996 and 1997 were generally normally distributed. Lateral flush consistently preceded terminal flush by several days, which produced skewed distributions for this trait (Table 2). An assumption imposed on regression analysis is that the residuals of the fitted regression follow a normal distribution. To test if the skewed lateral flush data interfered with QTL mapping, we analyzed both transformed [ARCSIN (SQRT)] and non-transformed data for *wlat*96 and *olat*96. Results showed that map positions were the same with both transformed and non-transformed data, and *F*-values differed very little. Because the effect was negligible, transformations were not performed on the remainder of the phenotypic data prior to QTL analysis. Clonal correlation coefficients among all traits were estimated by using PROC CORR (SAS 1989–1996) and were generally moderate to large (0.31–0.78) (Table 3).

Genotyping and linkage data

A previously constructed linkage map for Douglas-fir (Jermstad et al. 1998) was used to select 74 evenly distributed and informative RFLP markers for QTL mapping. Although there was a total of 224 clones with phenotypic data, only 190 clones (those with the highest number of ramets) were genotyped for QTL analysis.

QTL analysis

An all-marker, multiple regression method was used to estimate QTLs (Knott et al. 1997). Each linkage group was evaluated for one and two QTLs per linkage group (LG) at 5-centiMorgan (cM) intervals. For analyses conducted on the 12 individual traits (Table 1) two different models were tested: (1) 1 vs 0 QTLs per LG (3 degrees of freedom/*n*-1 degrees of freedom), and (2) 2 vs 0 QTLs per LG (6 degrees of freedom/*n*-1 degrees of freedom) (Knott et al. 1997). For each model, paternal, maternal, and paternal×maternal interaction effects were estimated. The proportion of phenotypic variance explained by each QTL was calculated as:

σ^2_p=[(reduced model SS/df)–(full model SS/df) /reduced model SS/df].

Thresholds of *F*-distribution probabilities p(*F*) for suggestive and significant QTL estimations were established at $p \leq 0.01$ and $p \leq 0.005$, respectively. The marker information on four linkage groups (4, 6, 9 and 11) did not meet 'full rank' criteria for regression analysis because segregation information was sub-optimal for one of the two parents. For QTLs detected on these LGs, effects could only be estimated for one parent, making it impossible to establish the effect of parental interaction. In such cases, the numerator degrees of freedom were reduced by 1.0, and the *F*-value probabilities were determined accordingly (Knott et al. 1997).

QTL by environment interactions

QTL×year interactions

QTL×year interactions were estimated by analyzing 2 years (1995 and 1996) of terminal bud flush data from each site (Table 1), with year as a fixed effect and a year×genotype interaction term included in the regression model. The 1997 data from the Washington site was not included, since the Oregon site clones were not measured for bud flush in 1997. We used all the data available from each site in the interaction analysis, 190 clones from Washington and 78 clones from Oregon. In these analyses, the 1 vs 0 QTL model (18 degrees of freedom/*n*-1 degrees of freedom) was tested at 5-cM intervals (Knott et al. 1997).

QTL×site interactions

Data from both sites for terminal bud flush in 1995 and 1996, and bud flush in 1998 were analyzed fitting site and site×genotype in-

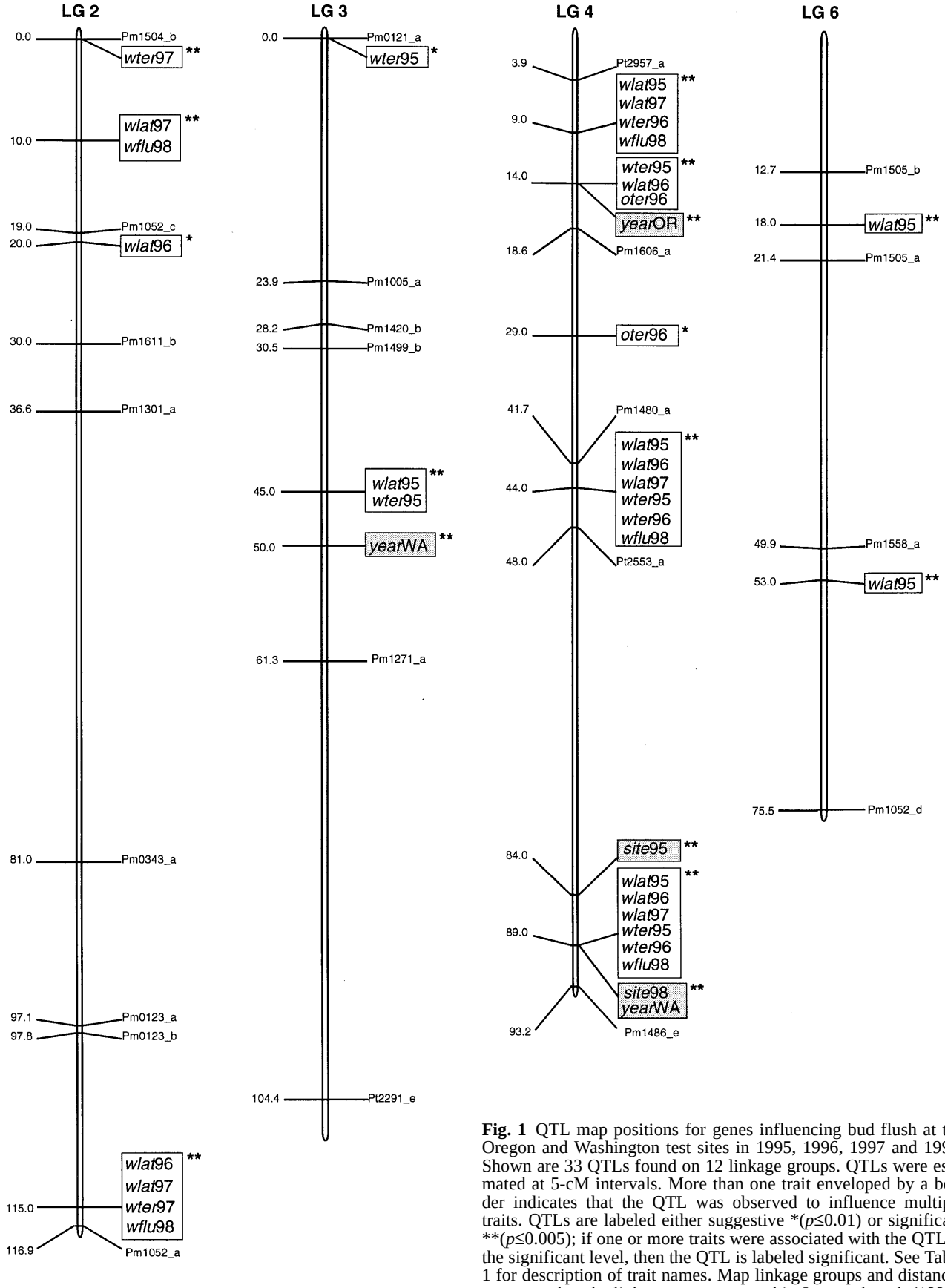

Fig. 1 QTL map positions for genes influencing bud flush at the Oregon and Washington test sites in 1995, 1996, 1997 and 1998. Shown are 33 QTLs found on 12 linkage groups. QTLs were estimated at 5-cM intervals. More than one trait enveloped by a border indicates that the QTL was observed to influence multiple traits. QTLs are labeled either suggestive *($p \leq 0.01$) or significant **($p \leq 0.005$); if one or more traits were associated with the QTL at the significant level, then the QTL is labeled significant. See Table 1 for description of trait names. Map linkage groups and distances correspond to the linkage map presented in Jermstad et al. (1998). Markers that were selected for use in the QTL analyses are shown

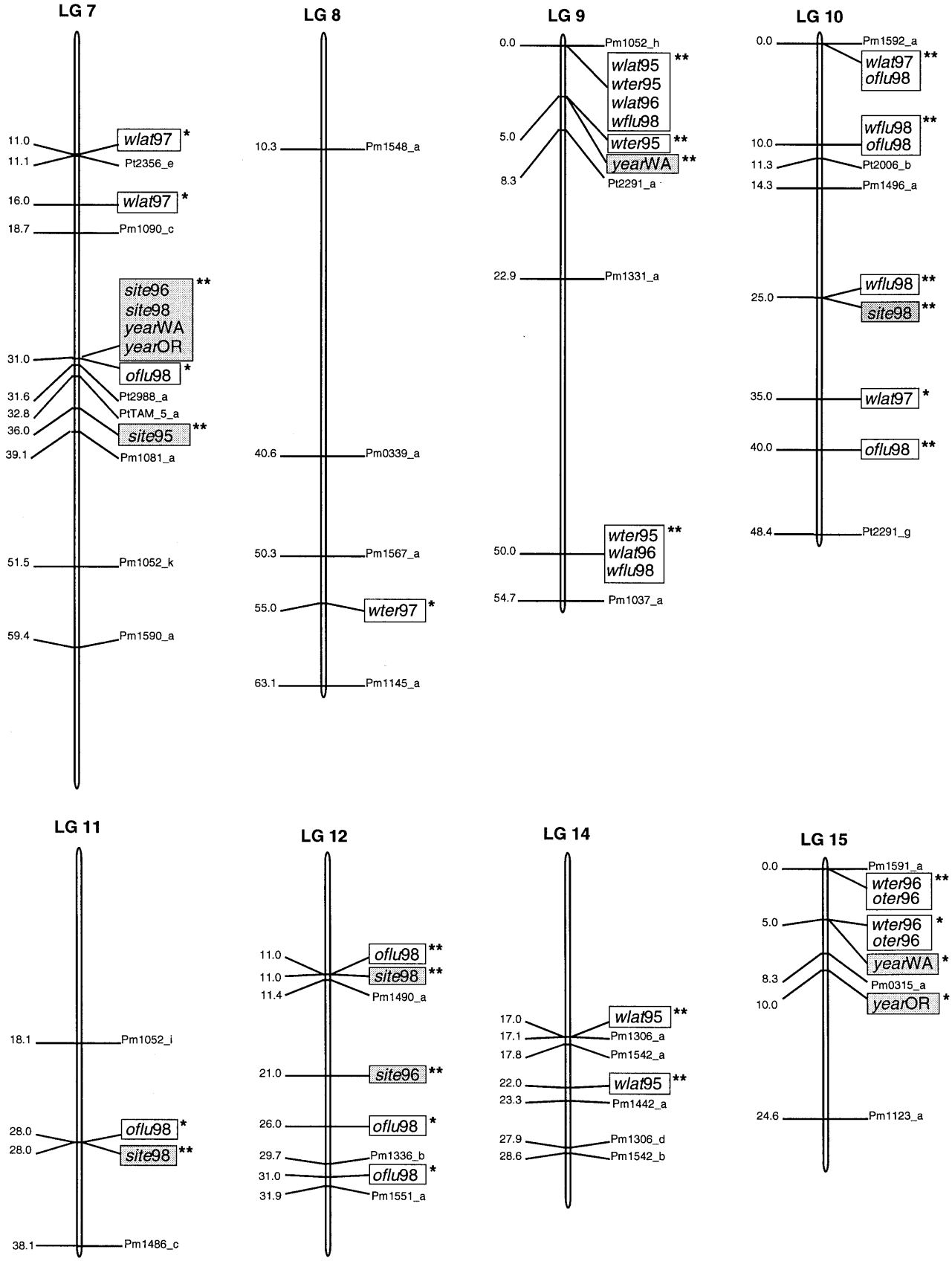

Fig. 1 (continued)

Table 4 Bud flush QTLs detected following the 1 versus 0 QTL model. Linkage group (LG) and map position (cM) are presented for each QTL along with *F*-values, paternal and maternal effects, paternal×maternal interaction effects, and the proportion of total phenotypic variance explained by the QTL. Standard errors for effects are presented in parentheses (see Table 1 for description of trait names)

Trait	LG	Map position (cM)	*F*-value	Pat. effect (SE)	Mat. effect (SE)	Pat.×mat. effect (SE)	Proportion var. (%)
Washington Site							
*wlat*95	3	45	4.71**	–0.09 (0.03)	0.01 (0.03)	0.01 (0.03)	5.6
*wlat*95	4	89	5.70**	–[a]	0.05 (0.05)	–	2.4
*wlat*95	9	0	5.71**	0.05 (0.02)	–	–	2.4
*wlat*96	4	89	10.22**	–	0.07 (0.02)	–	4.7
*wlat*96	9	0	6.91**	0.05 (0.02)	–	–	3.0
*wlat*97	2	115	4.28*	0.00 (0.03)	0.07 (0.05)	0.15 (0.05)	4.9
*wlat*97	4	89	6.22**	–	0.06 (0.03)	–	2.7
*wter*95	3	45	5.42**	–0.11 (0.03)	0.01 (0.03)	–0.01 (0.03)	6.6
*wter*95	4	89	5.56**	–	0.06 (0.03)	–	2.4
*wter*95	9	5	7.11**	0.07 (0.03)	–	–	3.1
*wter*96	4	89	7.45**	–	0.07 (0.03)	–	3.3
*wter*96	15	5	3.62*	0.01 (0.03)	–0.09 (0.04)	–0.09 (0.04)	4.0
*wter*97	8	55	4.19*	–0.10 (0.03)	0.01 (0.04)	–0.03 (0.04)	4.8
*wflu*98	2	115	4.47**	–0.05 (0.04)	0.09 (0.08)	0.28 (0.08)	5.2
*wflu*98	4	89	8.72**	–	0.13 (0.02)	–	3.9
*wflu*98	9	50	5.02*	0.10 (0.05)	–	–	2.1
*wflu*98	10	10	4.17*	0.00 (0.05)	0.16 (0.05)	0.01 (0.05)	4.8
Oregon Site							
*oflu*98	7	31	4.00*	–0.07 (0.09)	0.11 (0.09)	–0.31 (0.01)	9.7
*oflu*98	10	40	4.63**	–0.16 (0.14)	0.43 (0.14)	–0.09 (0.23)	11.5
*oflu*98	11	28	4.56*	–	0.19 (0.09)	–	4.1
*oflu*98	12	11	4.55 **	0.78 (0.08)	–0.23 (0.08)	–0.05 (0.08)	11.2

* $p \leq 0.01$, ** $p \leq 0.005$

[a] Markers on this linkage group provide information for only one parent. Regression is not 'full rank'

teraction terms (Table 1). The data were balanced with equal samples from each site (n=78). The 1 vs 0 QTL model (18 degrees of freedom/n-1 degrees of freedom) was tested at 5-cM intervals.

Results and discussion

Number, proportion of explained phenotypic variance, and effects of QTLs

Thirty three unique quantitative trait loci affecting spring bud flush were detected in this study (Fig. 1). This number is a synopsis of the 69 separate QTL inferences following all analyses (Tables 4 and 5). There were seven QTLs detected by both models that were counted only once. For example, the QTL for *wter*95 found on LG 3 at 45 cM was detected following both the 1 vs 0 QTL and the 2 vs 0 models, but is documented only once in Fig. 1. The remaining 62 QTLs that were detected for lateral and terminal bud flush in multiple years and at both sites were also documented in Fig. 1; however, QTLs sharing the same map position were enveloped by a border and counted as one unique QTL. Twenty two of the thirty three unique QTLs were estimated at the significant level ($p \leq 0.005$). In some cases, a significant QTL mapped to the same position as a suggestive QTL; consequently, this unique QTL was inferred as significant. The 33 unique QTLs mapped to 12 linkage groups.

Individual QTLs generally explained a small proportion of the phenotypic variance. Of the 69 individual QTL estimates, all but six each explained less than 8% of the total phenotypic variation for the trait analyzed. One QTL for *oflu*98 on LG 10, however, accounted for 11.5% of the phenotypic variance. The four QTLs detected for *oflu*98 explained the largest proportion of phenotypic variance for a single trait (36.5%), following the 1 vs 0 QTL model (Table 4). Assuming a moderate heritability (h^2=0.5) for spring bud flush, QTLs explaining 36.5% of the total phenotypic variation would account for approximately 73% of the total genetic variance. In general, QTLs detected at the Oregon site explained a larger percentage of the phenotypic variance than those detected at the Washington site. The sample of clones in Oregon was less than half (41%) the sample number in Washington, which could account not only for the reduction in number of QTLs detected at the Oregon site, but also in the increased proportion of explained phenotypic variance. Simulation studies have shown that, as sample size decreases, the power to detect QTLs is diminished, and the magnitude of effects is overestimated (Beavis 1995).

The estimated effects of QTLs can be partitioned into maternal, paternal, and maternal×paternal effects (Knott et al. 1997). The paternal and maternal effects reflect the magnitude and direction of influence that the alleles of a QTL have on a trait. The sign of the parental effect is the mean difference of effect of the two alleles inherited from the parent and reflects the direction of control that the allele inherited from the grandparent has in the F_2 progeny. More specifically, if an early flushing grandparent (high-scoring grandparent) transmits an allele to a parent that contributes to late flushing in the F_2 progeny instead of early flushing, then the sign of the effect for the parent is negative. For example, the QTL for *wter*95 on LG 3 (45 cM) (Table 4) has a negative paternal effect,

Table 5 Bud flush QTLs detected following the 2 versus 0 QTL model. Linkage group (LG) and map position (cM) are presented for each QTL along with *F*-values, paternal and maternal effects, paternal×maternal interaction effects, and the proportion of total phenotypic variance explained by the QTL. Standard errors for effects are presented in parentheses (see Table 1 for description of trait names)

Trait	LG	Map position (cM)	*F*-value	Pat. effect (SE)	Mat. effect (SE)	Pat.×mat. effect (SE)	Proportion var. (%)
Washington Site							
*wlat*95	4	9	2.93*	–[a]	–2.59 (1.08)	–	2.0
		44		–	1.42 (0.59)	–	
*wlat*95	6	18	3.36**	–	–0.02 (0.01)	–	1.2
		53		–	–0.05 (0.03)	–	
*wlat*95	14	17	3.35**	0.02 (0.01)	0.00 (0.01)	0.03 (0.01)	3.6
		22		0.01 (0.01)	0.00 (0.01)	0.03 (0.01)	
*wlat*96	2	20	3.05*	–0.02 (0.03)	–0.05 (0.02)	–0.03 (0.03)	6.1
		115		–0.02 (0.02)	0.06 (0.04)	0.09 (0.04)	
*wlat*96	4	14	7.30**	–	–3.19 (0.86)	–	6.2
		44		–	1.90 (0.52)	–	
*wlat*96	9	0	4.00**	0.05 (0.02)	–	–	3.1
		50		0.02 (0.02)	–	–	
*wlat*97	2	10	3.23**	–0.02 (0.04)	–0.06 (0.03)	–0.01 (0.04)	6.6
		115		0.01 (0.02)	0.10 (0.05)	0.14 (0.05)	
*wlat*97	4	9	3.21*	–	–3.00 (1.18)	–	2.3
		44		–	1.60 (0.65)	–	
*wlat*97	7	11	2.91*	0.03 (0.02)	0.00 (0.01)	–0.05 (0.02)	2.9
		16		0.03 (0.02)	0.00 (0.01)	–0.06 (0.02)	
*wlat*97	10	0	3.04*	–0.02 (0.02)	0.05 (0.02)	0.02 (0.03)	3.1
		35		–0.02 (0.02)	0.04 (0.02)	0.01 (0.02)	
*wter*95	3	0	2.82*	0.02 (0.02)	0.02 (0.07)	0.02 (0.04)	5.5
		45		–0.11 (0.03)	0.00 (0.05)	–0.02 (0.04)	
*wter*95	4	14	2.96*	–	–2.58 (0.11)	–	2.0
		44		–	1.56 (0.64)	–	
*wter*95	9	0	4.55**	0.05 (0.02)	–	–	3.6
		50		0.04 (0.03)	–	–	
*wter*96	4	9	4.29**	–	–3.74 (1.28)	–	3.4
		44		–	2.03 (0.70)	–	
*wter*96	15	0	3.62**	0.01 (0.01)	–0.04 (0.02)	–0.04 (0.02)	4.0
		5		0.01 (0.02)	–0.05 (0.02)	–0.05 (0.02)	
*wter*97	2	0	3.30**	0.03 (0.05)	–0.09 (0.03)	0.02 (0.06)	6.8
		115		0.04 (0.03)	0.09 (0.05)	0.13 (0.05)	
*wflu*98	2	10	3.52**	–0.01 (0.07)	–0.11 (0.05)	–0.08 (0.08)	7.4
		115		–0.05 (0.04)	0.14 (0.08)	0.03 (0.08)	
*wflu*98	4	9	6.20**	–	–7.20 (2.11)	–	5.2
		44		–	3.90 (1.15)	–	
*wflu*98	9	0	3.74**	0.07 (0.04)	–	–	2.8
		50		0.09 (0.05)	–	–	
*wflu*98	10	10	4.17**	0.00 (0.03)	0.09 (0.03)	0.01 (0.03)	4.8
		25		0.00 (0.02)	0.08 (0.02)	0.01 (0.02)	
Oregon Site							
oter96	4	14	2.89*	–	0.03 (0.02)	–	2.4
		29		–	0.04 (0.02)	–	
oter96	15	0	3.34*	–0.00 (0.01)	0.03 (0.02)	–0.05 (0.02)	8.4
		5		–0.00 (0.02)	0.03 (0.02)	–0.07 (0.03)	
oflu98	10	0	4.63**	–0.05 (0.04)	0.14 (0.04)	–0.02 (0.45)	11.5
		10		–0.06 (0.05)	0.17 (0.05)	–0.03 (0.07)	
oflu98	12	26	2.86*	0.14 (0.06)	–1.40 (0.65)	0.12 (0.90)	10.0
		31		0.12 (0.05)	1.20 (0.64)	–0.27 (0.95)	

* $p \leq 0.01$, ** $p \leq 0.005$

[a] Markers on this linkage group provide information for only one parent. Regression is not 'full rank'

meaning that the allele transmitted to the progeny through the male parent by the high-scoring paternal grandfather does not express early bud flush as predicted. The majority of the parental effects reported in Tables 4 and 5 are small and of positive effect, and the number of negative effects were about equal for both parents. These results were expected because, even though the grandparent pairs were chosen to be early and late for the date of bud flush, they were not inbred for these traits and would not be fixed for alternative alleles at all the QTLs controlling bud flush.

The magnitude of the maternal×paternal effect reflects additive versus non-additive gene action, with greater deviation from zero indicating stronger non-additive gene action (Knott et al. 1997). Estimates of the maternal×paternal effect for most QTLs differed little from zero, suggesting that bud flush is controlled predominantly by genes that are additive in effect. Small non-additive effects were found on LG 2 (115 cM) in the Washington-site analyses (Tables 4 and 5) and on LG 7(31 cm) in the Oregon-site analyses (Table 4). These results are consistent with common garden experiments which have

Table 6 Terminal bud flush QTL×year interactions detected at the Washington and Oregon test sites. A QTL×year interaction term was fitted into the interval mapping regression model (Knott et al. 1997). Map position, *F* values, and the proportion of variance (Var.%) explained by a QTL are shown

LG	Washington (*year*WA)			LG	Oregon (*year*OR)		
	Map position (cM)	*F*-value	Var. (%)		Map position (cM)	*F*-value	Var. (%)
3	50	3.5**	3.9	–	–	–	–
4	89	6.2**	2.7	4	14	2.6**	3.7
7	31	2.0*	1.6	7	31	3.1**	7.6
9	5	5.8**	2.5	–	–	–	–
15	5	2.0*	1.6	15	10	2.0*	3.7

* $p \leq 0.01$, ** $p \leq 0.005$

Table 7 Terminal bud flush QTL×site interactions detected for each of 3 years. A QTL×site interaction term was fitted into the interval mapping regression model (Knott et al. 1997). Map position, *F*-values, and the proportion of variance (Var.%) explained by a QTL are shown

1995 (*site*95)				1996 (*site*96)				1998 (*site*98)			
LG	Map position (cM)	*F*-value	Var. (%)	LG	Map position (cM)	*F*-value	Var. (%)	LG	Map position (cM)	*F*-value	Var. (%)
4	84	4.9**	4.8	–	–	–	–	4	89	2.1*	1.3
7	36	2.8**	6.5	7	31	3.4**	8.4	7	31	3.6**	8.5
–	–	–	–	–	–	–	–	10	25	4.7**	11.6
–	–	–	–	–	–	–	–	11	28	2.9**	2.2
–	–	–	–	12	21	2.5**	5.7	12	11	3.9**	9.4

* $p \leq 0.01$, ** $p \leq 0.005$

shown that the timing of bud flush in Douglas-fir is largely additive in effect, while only a small percent is due to genes of non-additive effect (Rehfeldt 1983; El-Kassaby and Park 1993).

Estimation of QTLs in multiple years

QTLs controlling spring bud flush were estimated in 4 different years (1995, 1996, 1997 and 1998) at the Washington site and 3 different years (1995, 1996 and 1998) at the Oregon site (Tables 4 and 5). In several cases at the Washington site, QTLs controlling the same trait were detected at the same map position for multiple years. A good example of this is found on LG 4 at 0 cM, 4 cM, and 89 cM; other examples can be found on LGs 2 and 9 (Fig. 1). These results support the conclusions of Li and Adams (1993) that a similar suite of genes control the timing of spring bud flush through different developmental stages in the life of the tree. However, QTLs controlling the same bud flush traits were not detected at the same map position in multiple years at the Oregon site. This may have been because of the low statistical power of detection due to the smaller population sample at this site.

To further explore the temporal patterns of QTL expression, QTL×year interactions were estimated at each site (Table 6). Five QTL×year interactions were detected at the Washington site (LGs 3, 4, 7, 9 and 15) and three QTL×year interactions were detected at the Oregon site (LGs 4, 7 and 15) (Fig. 1). A QTL×year interaction was detected on LG 7 (31 cM) at each site. All of the QTL×year interactions were found either at exactly the same map locations as individual QTL estimates, or in close proximity to individual QTL estimates (LG 3, 50 cM, and LG 15, 10 cM).

Given that the power to detect QTLs in any one year was not exceptionally high in this experiment, the repeated detection observed across years and the small number of QTLs with year interactions, suggest that most genes controlling the onset of spring bud flush in Douglas-fir are expressed annually.

Estimations of QTLs at multiple sites

Another objective of this study was to determine if the same QTLs could be detected in different test environments. The clonal propagation of the mapping-population progeny enabled the establishment of the two replicated test sites. In general, QTLs controlling bud flush were detected at genomic locations for either the Washington site or the Oregon site, but not for both sites at the same locations. Exceptions were found on LGs 10 and 15 where a bud flush QTL was found at the same location from both test sites. These results suggest that different QTLs are expressed in different environments.

To further test this hypothesis, QTL×site interactions were estimated (Table 7). Nine QTL×site interactions were detected, eight of which were estimated at a significant probability level ($p \leq 0.005$), and six of which mapped to locations where QTLs had previously been detected (Fig. 1). Two of the QTLs×site interactions were estimated at the same map position as two QTL×year interactions (LG 7, 31 cM).

Although the power to detect QTLs at the Oregon site is limit, the general result of these data suggest that there are many genes controlling bud flush in Douglas-fir and that different genes are expressed in different environments.

Conclusions

QTLs have been estimated for a number of traits in forest trees and most QTLs detected individually explain a small percent of the total phenotypic variation (Sewell and Neale 2000). For Douglas-fir, several QTLs controlling the timing of spring bud flush were detected in this study, each explaining a relatively small proportion of the total phenotypic variation. The power to detect the QTLs controlling a trait, and to accurately estimate the magnitude of their effects, is dependent upon the size of the mapping population, the marker information, and the ability to accurately measure the trait. Thus far, the only other reports of QTLs for the timing of bud flush in forest trees are for poplar (Bradshaw and Stettler 1995; Frewen et al. 2000). Frewen et al. (2000) measured the timing of spring bud flush in 346 clonally replicated F_2 hybrid poplar progeny planted at a single and uniform test site in Corvallis, Oregon. Six unique QTLs were estimated, in which each of five individually explained less than 10% of the phenotypic variation. Although the small proportion of variance explained by these individual QTLs was comparable to the results we obtained in Douglas-fir, substantially fewer QTLs for bud flush were detected in the poplar study than for Douglas-fir, even though a larger mapping population was employed. Perhaps a larger number of QTLs influence the timing of spring bud flush in Douglas-fir, but, more likely, the reasons for the large difference in the number of bud flush QTLs detected in these two species are due to the experimental design. In the Douglas-fir experiment, phenotypic measurements were made on two bud flush traits (lateral and terminal) at two geographic locations over multiple years and were estimated following two models. Thus, if we were to count the number of QTLs controlling the timing of bud flush in just 1 year at one site following only the 1 vs 0 QTL model, the highest number of QTLs detected per trait would be four (*wflu*98 and *oflu*98; Table 4). It is unknown if the number of QTLs for the timing of bud flush in poplar would be increased if measurements at multiple sites and in multiple years were included in the analysis.

To-date, this is the first report of bud flush QTL estimation over multiple years. The repeated detection of QTLs in multiple years (at the Washington site) and few QTL×year interactions suggest that several of the genes controlling the timing of bud flush in Douglas-fir are repeatedly expressed over early developmental stages. This is congruent with earlier genetic studies reporting that a similar set of genes control bud flush in both seedling- and sapling-aged trees (Li and Adams 1993). Growth traits in forest trees have been analyzed for QTLs over multiple growing seasons. Plomion et al. (1996) detected some repeatability of QTLs for components of growth across shoot cycles in Maritime pine (*Pinus pinaster*), and Verhaegen et al. (1997) showed that QTLs for height:diameter ratio were repeatedly expressed over developmental stages in a *Eucalyptus* hybrid. In contrast, repeatability over multiple growing seasons was not detected for growth traits in loblolly pine (*Pinus taeda* L.) (Kaya et al. 1999) or in *Populus* hybrids (Bradshaw and Stettler 1995).

Our analyses detected very little overlap of QTLs controlling bud flush at the two test sites and several QTL×site interactions, suggesting that the QTLs controlling the timing of bud flush are differentially expressed in different environments. However, the sample data in this study was substantially smaller at the Oregon site and additional experimentation is needed to verify our results. We have recently conducted an experiment using a large family of clones (n=475) to estimate QTLs×site interactions, and QTLs responding to specific environmental cues, such as photoperiodicity, temperature and moisture. Replicated progeny have been subjected to varying winter chill and heat sums in controlled environments for an estimation of the QTLs influencing the timing of bud flush, while photoperiod and moisture availability have been manipulated to estimate QTLs influencing the timing of bud set. These studies will provide a knowledge of the location and organization of phenology QTLs that respond to environmental factors, and enhance our understanding of the role that the environment plays in the induction and cessation of the growth cycle in Douglas-fir.

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