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Mapping of quantitative trait loci controlling adaptive traits in coastal Douglas-fir. II. Spring and fall cold-hardiness

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Abstract Quantitative trait loci (QTLs) affecting fall and spring cold-hardiness were identified in a three-generation outbred pedigree of coastal Douglas-fir [*Pseudotsuga meniziesii* (Mirb.) Franco var. *menziesii*]. Eleven QTLs controlling fall cold-hardiness were detected on four linkage groups, and 15 QTLs controlling spring cold-hardiness were detected on four linkage groups. Only one linkage group contained QTLs for both spring and fall cold-hardiness, and these QTLs tended to map in close proximity to one another. Several QTLs were associated with hardiness in all three shoot tissues assayed in the spring, supporting previous reports that there is synchronization of plant tissues during de-acclimatization. For fall cold-hardiness, co-location of QTLs was not observed for the different tissues assayed, which is consistent with previous reports of less synchronization of hardening in the fall. In several cases, QTLs for spring or fall cold-hardiness mapped to the same location as QTLs controlling spring bud flush. QTL estimations, relative magnitudes of heritabilities, and genetic correlations based on clonal data in this single full-sib family, supports conclusions about the genetic control and relationships among cold-hardiness traits observed in population samples of Douglas-fir in previous studies.

Keywords QTL mapping · RFLP · Freeze testing · Tissue damage

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Introduction

The ability of perennial plants to withstand unseasonably cold temperatures without sustaining damage is a critical adaptive trait. Damage often occurs in the spring after dormancy is released or in the fall before winter acclimation is complete. In young plants, cold damage causes death or decreased growth, resulting in reduced ability to compete with surrounding flora (Timmis et al. 1994). Cold-hardiness is not only critical to the yield of perennial food crops but also to the sustained growth of forest trees. In established trees, cold damage can cause stem defects and a reduction in wood quality (Schermann et al. 1997). Several genecological studies have been conducted on cold-hardiness in forest trees, including coastal Douglas-fir [*Pseudotsuga meniziesii* (Mirb.) Franco var. *menziesii*] (Aitken and Hannerz 2000.).

In Douglas-fir, the ability to resist cold damage in different seasons is influenced by genotype and by various environmental cues. Spring cold-hardiness is under strong genetic control and is responsive to temperature, whereas fall cold-hardiness is less heritable and is more strongly influenced by photoperiod, available moisture, and other environmental factors (Greer et al. 1989; Aitken and Adams 1996, 1997; Johnsen et al. 1996; Aitken and Hannerz 2000).

Estimated genetic correlations between fall and spring cold-hardiness traits in Douglas-fir are usually negative, but only weak to moderate in magnitude, suggesting that cold-hardiness is largely under the control of different genes in the two seasons (Aitken and Adams 1995; O'Neill 1999; Anekonda et al. 2000b). Also, estimated genetic correlations in cold-hardiness between different

shoot tissues (i.e., bud, needle and stem) in this species are generally stronger in the spring than in the fall (Aitken and Adams 1996, 1997; O'Neill 1999). This suggests that there is more overlap in genes controlling the cold-hardiness of different tissues in the spring than in the fall. Spring cold-hardiness has a strong negative correlation with the timing of spring bud flush in Douglas-fir (Aitken and Adams 1997; O'Neill 1999). Early flushing genotypes are more susceptible to frost damage than late-flushing genotypes, even prior to the emergence of new tissue.

Fall cold-hardiness in Douglas-fir is under weak genetic control compared to spring cold-hardiness (White 1987; Aitken and Adams 1997; Anekonda et al. 2000b) and estimated genetic correlations between fall cold-hardiness and the timing of bud set are generally weaker than those between spring cold-hardiness and the timing of spring bud flush (Aitken and Adams 1995, 1996). This is because the speed of acclimatization is differentially influenced by environmental factors such as summer moisture, which can vary widely between years and among microenvironments within sites. Drought may hasten shoot acclimatization while a favorable moisture regime in the summer may foster second flushing in young Douglas-fir, delaying acclimatization in shoots that have a second flush (Anekonda et al. 1998).

Measurement of cold damage in the field is wholly dependent upon weather conditions (i.e., occurrence of frost events) and is, consequently, difficult to obtain. Also, it is difficult to obtain equal treatment of genotypes in the field due to microsite variation and tree-to-tree variation in exposure. In the late seventies, Rehfeldt (1979) employed artificial freeze testing for studying cold-hardiness and delineating seed zones in interior Douglas-fir (var. *glauca*) populations. More recently, Aitken and Adams (1996, 1997) and O'Neill (1999) used artificial freeze testing to study genetic variation in cold-hardiness among families in two low-elevation coastal Douglas-fir populations from western Oregon. They found that the mean injury scores of families after artificial freeze testing were strongly correlated with the mean injury scores of the same families after natural frost events. They also concluded that using artificial freeze testing was an efficient and reliable method to rank families for cold-hardiness in tree-breeding programs.

We utilized artificial freeze testing to map quantitative trait loci (QTLs) for spring and fall cold-hardiness in a three-generation mapping population of coastal Douglas-fir. Although this cross was originally designed to maximize the segregation in timing of spring bud flush, we anticipate that genes influencing other adaptive traits, such as spring cold-hardiness, are segregating as well. A multiple marker interval-mapping method (Knott et al. 1997) was used to estimate QTLs. We report 11 unique QTLs on four linkage groups (LG) influencing fall cold-hardiness and 15 unique QTLs on four LGs influencing spring cold-hardiness.

Materials and methods

Mapping population and genetic test plantations

The mapping population is a Douglas-fir three-generation outbred pedigree that is segregating for the timing of bud flush (Jermstad et al. 1998). Grandparent pairs were selected based on the timing of the 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 long-term test sites at Twin Harbors, Washington, and Turner, Oregon. An incomplete randomized block design was used with four blocks per site, and clones were planted in 3-tree row plots. Clones from both sites were analyzed for the estimation of bud flush QTLs (Jermstad et al. 2001); however, in the present study, only clones from the Washington site were analyzed. In the fall of 1997, there were 224 clones growing at the Washington site with an average of eight ramets per clone. 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.

Lateral shoot-tip sampling

Four 5-cm-long lateral shoot tips were harvested from each sampled ramet in the fall of 1996 (October 7) and in the spring of 1997 (April 8) prior to bud flush. Not all of the clones that had been genotyped (190) had sufficient numbers of ramets for sampling. Furthermore, sampling had to be restricted in order to minimize damage to the trees. Thus, in the fall, 1–2 ramets of each of 186 clones were sampled in two of the four blocks (mean number of total ramets per clone=3.5). In the spring, 1–2 ramets from each of 171 clones included in the fall collection were sampled in the remaining two blocks (mean number of total ramets per clone=3.3).

Cold treatment

Artificial freezing for the assessment of cold-hardiness in Douglas-fir is described in Aitken and Adams (1997) and Anekonda et al. (2000a). Shoot tips were wrapped in damp cheesecloth and aluminum foil prior to being stored overnight at –2°C. The temperature was then slowly decreased until the test temperature was attained, where it was held constant for 1 hour. For freeze-testing in the fall, the samples were subjected to –9°, –11°, –13° and –15°C and for freeze-testing in the spring, the samples were subjected to –12°, –14°, –16° and –18°C. The samples were then stored at 2°C overnight then held for seven days in the dark at room temperature to allow cold-injury symptoms to develop. Preliminary sampling and freeze-testing were conducted on tissue collected from the test site 1 week prior to the experiment to empirically determine the appropriate freeze test temperatures that would inflict a range of mean injury to shoot tissues, ranging from high to low.

Scoring of freeze injury

Bud, needle, and stem (phloem and cambium) tissue were each evaluated for freeze-injury symptoms by visually estimating the

Table 1 Description of cold-hardiness traits, year of measurement, and names of traits

Trait Description	Year	Trait Name
Fall cold injury (buds)	1996	*fch*_b
Fall cold injury (needles)	1996	*fch*_n
Fall cold injury (stems)	1996	*fch*_s
Spring cold injury (buds)	1997	*sch*_b
Spring cold injury (needles)	1997	*sch*_n
Spring cold injury (stems)	1997	*sch*_s

degree of necrosis to the exposed tissue (Anekonda et al. 2000a). A scale from 1 to 10 was used to score injury, with 10 denoting severe injury. The highest and lowest test temperatures in the fall and spring produced injury levels that were either too high or too low, respectively, to allow for the detection of clonal differences. Thus, only freeze-testing scores at the two intermediate temperatures were utilized.

Cold-injury traits and estimated clonal correlations

The cold-injury scores for the two intermediate temperatures tested in the spring and fall were strongly correlated among clones; therefore, scores for the three tissues in each season were averaged so that a total of six cold-injury traits (3 shoot tissues×2 seasons) were evaluated in this study (Table 1). The range and means of injury scores, the broad-sense heritabilities, and the genetic correlations among cold-injury traits were estimated as described in Anekonda et al. (2000b). Analyses of variance showed that variation among clonal means for all six cold-injury traits was significant ($p<0.01$) and considerable. For example, the range among clones in mean percent injury to stems was 25–95 in the fall and 43–90 in the spring. Other traits had similar ranges among clones (Anekonda et al. 2000b). Clonal correlations among the mean cold-injury scores for the six traits were estimated using PROC CORR (SAS Institute 1989–1996). Data for the timing of spring bud flush were collected at the Washington site (Jermstad et al. 2000) during the same years as injury scores from freeze-testing were collected. Thus, clonal correlations among the six cold-injury traits and the spring bud flush scores (*wter*96 and *wter*97) were also estimated. The bud flush scores were based on the proportion of ramets within each clone with a flushed terminal bud on a single Julian date in the spring of both 1996 and 1997. Therefore, early flushing genotypes had high bud flush scores. A full description of the scoring technique used for spring bud flush is presented in Jermstad et al. (2000).

Linkage map and QTL estimations

A previously constructed linkage map for Douglas-fir (Jermstad et al. 1998) was used to select 74 evenly distributed and informative markers for QTL mapping (Jermstad et al. 2001). QTL analysis was performed using the multiple-marker method described in Knott et al. (1997) and Jermstad et al. (2001). Clonal means of cold-injury scores, linkage data, and RFLP segregation data were analyzed for the estimation of QTLs on 184 clonal progeny for the fall and 170 clonal progeny for the spring. QTLs were estimated at 5-cM intervals following a 1 vs 0 QTL model (3 degrees of freedom/*n*-1 degrees of freedom) and a 2 vs 0 QTL model (6 degrees of freedom/*n*-1 degrees of freedom). Thresholds of *F*-distribution probabilities p(*F*) for suggestive and significant QTL estimations were established at $p\leq0.01$ and $p\leq0.005$, respectively. The marker information on two linkage groups (4 and 6) did not meet 'full rank' criteria for regression analysis because segregation information was sub-optimal for one of the two parents. In these cases, the numerator degrees of freedom were reduced by 1.0 and *F*-value probabilities were determined accordingly (Knott et al. 1997). The proportion of explained variance, the paternal and maternal effect, plus the paternal×maternal interaction effect, were estimated and reported. 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*].

Results and discussion

Number, proportion of phenotypic variance, and effects of QTLs

Eleven unique QTLs controlling fall cold-hardiness and 15 unique QTLs controlling spring cold-hardiness were detected in this experiment (Fig. 1). These numbers reflect a summary of the 31 separate QTLs detected following both models and for all tissue-types tested (Tables 2 and 3). There was one case in which a QTL was detected following both the 1 vs 0 QTL and the 2 vs 0 QTL models (*fch*_s on LG 8, 5 cM); therefore, only 30 QTLs were documented in Fig. 1. When QTLs for different tissues were mapped to the exact same position, they were enveloped in a box and counted as one unique QTL. There were four such cases in which a QTL was estimated for more than one spring trait: *sch*_b and *sch*_s on LG 1 (98 cM), *sch*_b and *sch*_n on LG 6 (43 cM), *sch*_b and *sch*_s on LG7 (26 cM), and *sch*_b and *sch*_n on LG 7 (56 cM). Fourteen of the fifteen QTLs controlling spring cold-hardiness and nine of the 11 QTLs controlling fall cold-hardiness were detected at the significant probability level ($p\leq0.005$). When more than one QTL was detected at a map position, only one QTL needed to be detected at the significant level for the QTL to be inferred as significant. QTLs for spring and fall cold-hardiness were detected on seven linkage groups: fall cold-hardiness QTLs were detected on LGs 2, 4, 6 and 8, and spring cold-hardiness QTLs were detected on LGs 1, 6, 7 and 10.

Linkage group 6 was the only LG that contained QTLs for both fall and spring cold-hardiness, with the fall cold-hardiness QTLs being detected within 5 cM of the spring cold-hardiness QTLs. Considering that QTL map locations are statistical estimations, and because QTLs were estimated at 5-cM intervals, it is plausible that any two closely mapped QTLs are the same QTL acting on more than one trait. Several of the QTLs for fall and spring cold-hardiness located on LGs 1, 2, 6, 7 and 10 are located within 5 cM of each other; therefore, it is feasible that these QTLs are the same genes controlling different cold-hardiness traits.

QTLs for fall cold-hardiness generally explained a small proportion of the phenotypic variance with an overall average of 4.1%. The two QTLs detected from needle tissue on LG 2 explained the largest proportion of phenotypic variance (6.8%) (Table 3). QTLs for spring cold-hardiness generally explained a slightly larger proportion of the phenotypic variance than those for fall cold-hardiness, ranging from 1.4 to 9.8%, with an overall average of 5.8%. Estimated narrow-sense heritabilities (Aitken

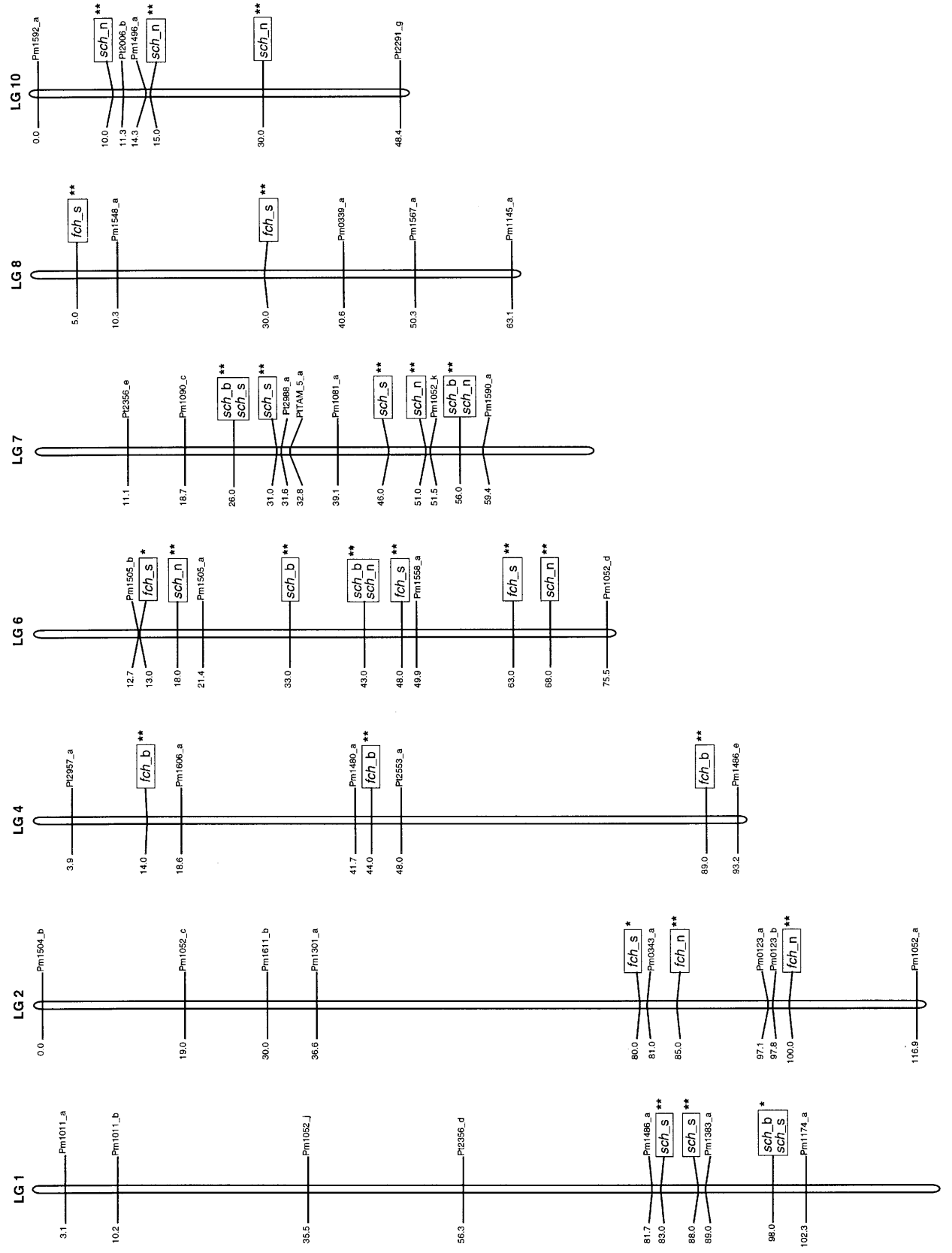

Fig. 1 QTL map positions for genes influencing fall and spring cold-hardiness. Shown *within boxes* are the positions of 11 unique QTLs for fall cold-hardiness and 15 unique QTLs for spring cold-hardiness. QTLs were estimated at 5-cM intervals. QTLs are labeled either suggestive *($p \leq 0.01$) or significant **($p \leq 0.005$); 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

Table 2 QTLs detected controlling fall and spring cold-hardiness following the 1 vs 0 QTL model. Shown are linkage group (LG) and map position, *F*-values for the model, maternal, paternal and maternal×paternal interaction effects, and the proportion of the total phenotypic variance explained by each QTL. Standard errors for effects are shown 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. (%)
*fch*_b	4	89	9.25**	–[a]	–0.436 (0.143)	–	4.3
*fch*_s	2	80	3.75*	–0.163 (0.104)	–0.291 (0.105)	0.121 (0.107)	4.3
*fch*_s	6	13	4.79*	–	0.792 (0.362)	–	2.0
*fch*_s	8	5	3.78*	0.554 (0.211)	0.335 (0.212)	–0.440 (0.433)	4.4
*sch*_b	1	98	3.66*	–0.057 (0.545)	0.290 (0.088)	–0.156 (0.591)	4.5
*sch*_n	6	18	7.72**	–	–0.992 (0.357)	–	3.8
*sch*_n	10	15	5.60**	0.025 (0.114)	0.367 (0.114)	–0.260 (0.115)	7.5
*sch*_s	1	98	3.65*	0.836 (0.403)	0.158 (0.065)	–0.313 (0.437)	4.5
*sch*_s	7	26	4.47**	0.027 (0.078)	0.010 (0.067)	–0.322 (0.089)	5.8

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

[a] Markers on this linkage group provide information for only one parent. The regression model is not "full rank"

Table 3 QTLs detected controlling fall and spring cold-hardiness following the 2 vs 0 QTL model. Shown are linkage group (LG) and map position, *F*-values for the model, paternal, maternal and paternal×maternal interaction effects, and the proportion of total phenotypic variance explained by each QTL. Standard errors for effects are shown 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. (%)
*fch*_b	4	14	5.23**	–[a]	0.002 (0.062)	–	4.4
		44		–	–12.0 (3.71)	–	
*fch*_n	2	85	3.69**	–0.416 (0.178)	–0.150 (0.076)	0.531 (0.193)	6.8
		100		0.357 (0.170)	–0.111 (0.056)	–0.596 (0.249)	
*fch*_s	6	48	4.79**	–	0.139 (0.064)	–	2.0
		63		–	0.188 (0.086)	–	
*fch*_s	8	5	3.78**	0.149 (0.057)	0.090 (0.057)	–0.052 (0.052)	4.4
		30		0.245 (0.096)	0.148 (0.094)	–0.143 (0.140)	
*sch*_b	6	33	3.47**	–	–0.145 (0.078)	–	1.4
		43		–	–0.178 (0.095)	–	
*sch*_b	7	26	3.07*	0.031 (0.057)	0.080 (0.119)	–0.540 (0.057)	5.8
		56		0.029 (0.053)	0.082 (0.117)	0.496 (0.169)	
*sch*_n	6	43	7.72**	–	–0.162 (0.058)	–	3.8
		68		–	–0.267 (0.096)	–	
*sch*_n	7	51	4.68**	0.117 (0.078)	–10.34 (6.04)	–17.11 (7.76)	9.8
		56		0.105 (0.070)	11.57 (6.58)	20.44 (9.34)	
*sch*_n	10	10	5.60**	0.017 (0.075)	0.242 (0.075)	–0.214 (0.095)	7.5
		30		0.013 (0.060)	0.192 (0.060)	–0.136 (0.060)	
*sch*_s	1	83	3.19**	0.318 (0.162)	0.319 (0.191)	1.84 (0.944)	6.2
		88		0.288 (0.147)	–0.190 (0.190)	–2.16 (1.04)	
*sch*_s	7	31	4.21**	0.014 (0.034)	–0.181 (0.137)	–0.559 (0.165)	8.7
		46		0.014 (0.035)	0.245 (0.128)	0.316 (0.153)	

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

[a] Markers on this linkage group provide information for only one parent. The regression is not "full rank"

and Adams 1996, 1997; O'Neill 1999) and broad-sense heritabilities (Anekonda et al. 2000b) for cold injury are typically about twice as great in the spring than in the fall. The mean broad-sense heritabilities for cold injury traits in the fall and spring were estimated at 0.17 and 0.31, respectively (Anekonda et al. 2000b).

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 the 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 a cold-susceptible grandparent (high-scoring grandparent) transmits an allele to a parent that contributes to cold-hardiness in the F_2 progeny instead of cold-susceptibility, then the sign of the effect for the parent is negative. An example can be seen on LG 2 (100 cM) in which the QTL for *fch*_n has a negative maternal effect (Table 3), indicating that the allele transmitted from the cold-susceptible (early flushing) paternal grandfather confers hardiness in the F_2 progeny rather than susceptibility to cold temperatures. Thirty

one percent (31%) of the loci detected show this type of inheritance, indicating that the genes controlling these two traits may not be co-segregating as much as expected. The pedigree was designed for segregation in the timing of the spring bud flush. Since trees that flush earlier are more susceptible to early cold injury, we expect early flushing grandparents to also be less hardy. The largest parental effects were maternal and found on LG 7 for *sch*_n (-10.34 and 11.57; Table 3).

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). Many of the QTLs detected for cold-hardiness appear to be additive in effect. Although several QTLs had maternal×paternal effects slightly greater than zero, the largest non-additive effects were found for spring cold-hardiness in needle and stem tissue on LGs 7 and 1, respectively (Table 3).

We know of only one previous report on the mapping of QTLs for cold-hardiness in forest trees. Byrne et al. (1997) mapped two QTLs for fall cold-hardiness in a large family of *Eucalyptus nitens* using single-factor analysis. The two QTLs were located on the same linkage group and accounted for 7.7% and 10.7% of the total phenotypic variation. We have estimated that several more QTLs are responsible for cold-hardiness in Douglas-fir than were estimated in *Eucalyptus*, but it is too early to conclude the number of genes controlling this trait or how many, if any, are conserved across taxa.

Comparison of QTLs for cold-hardiness across shoot tissues

In all cases, QTLs detected for fall cold-hardiness were unique to individual tissues, while QTLs detected for spring cold-hardiness were frequently associated with more than one tissue (Fig. 1). For example, QTLs for both bud and stem spring cold-hardiness were located on LG 1 (98 cM) and LG 7 (26 cM), and QTLs for both bud and needle tissue cold-hardiness were located on LG 6 (43 cM) and LG 7 (56 cM). This overlap of QTLs controlling cold-hardiness across tissues in the spring is consistent with earlier genetic studies in Douglas-fir that suggest a synchronization of de-acclimation among tissues in the spring (Aitken and Adams 1996, 1997; O'Neill 1999).

The genetic correlations among cold-injury scores in different shoot tissues within a season were mostly positive and moderate to strong (mean fall $|r_g|$=0.69; mean spring $|r_g|$=0.67), while correlations between similar tissue in different seasons were weak to moderate and mostly negative (Anekonda et al. 2000b). Clonal correlations (r_c) among the various cold-injury traits (Table 4), followed patterns expected from earlier genetic studies of cold-hardiness in Douglas-fir and from genetic correlations estimated with the same clonal materials (Aitken and Adams 1996, 1997; O'Neill 1999; Anekonda et al. 2000b) (Table 4). Fall cold-injury traits were usually negatively, but always weakly, correlated to spring cold-injury traits (mean $|r_c|$=0.010), while correlations in cold-injury traits among tissues measured in the same season were moderately and consistently positive (mean fall $|r_c|$=0.61; mean spring $|r_c|$=0.45).

Table 4 Clonal correlation coefficients among cold-injury traits and spring bud flush scores. See Table 1 for description of trait names

Trait	*fch*_b	*fch*_n	*fch*_s	*sch*_b	*sch*_n	*sch*_s
*fch*_b	–	0.55	0.60	–0.16	0.03	–0.05
*fch*_n		–	0.69	–0.10	0.12	–0.06
*fch*_s			–	–0.25	0.01	–0.13
*sch*_b				–	0.42	0.52
*sch*_n					–	0.41
*sch*_s						–
*wter*96	–0.27	–0.20	–0.25	0.51	0.27	0.42
*wter*97	–0.14	–0.14	–0.19	0.47	0.37	0.46

Comparison of QTLs for cold-hardiness and spring bud flush

There were six LGs on which QTLs for both cold-hardiness and spring bud flush were detected (LGs 6, 7, 10, for spring cold-hardiness and LGs 2, 4, 6, 8 for fall cold-hardiness) (Fig. 1; Jermstad et al. 2000). Three spring cold-hardiness QTLs were co-located with QTLs for spring bud flush on LG 6 (18 cM), LG 7 (31 cM) and LG 10 (10 cM), which is consistent with the moderately strong positive correlations between early spring bud flush (1996 and 1997) and spring cold injury (mean $|r_c|$=0.42) (Table 4) and with earlier reports (Aitken and Adams 1997; O'Neill 1999; Aitken and Hannerz 2000). Positive correlations between spring cold injury and bud flush scores were expected because clones with higher proportions of ramets with flushed buds midway in the flushing period have, on average, an earlier bud flush and, thus, shoots that are more sensitive to frost damage in early spring.

We observed co-location of QTLs for fall cold-hardiness and spring bud flush at 3 locations on LG 4 (14 cM, 44 cM and 89 cM). The QTLs at 44 cM and 89 cM for bud flush were detected in multiple years (Jermstad et al. 2001). This overlap of QTLs controlling fall cold-hardiness and spring bud flush (1996 and 1997) is consistent with the weak clonal correlations (mean $|r_c|$=0.20) estimated in this population (Table 4) and with the moderate correlations O'Neill (1999) found between these traits in 7 year-old saplings. Complex associations are characteristic among adaptive traits involving growth rhythm and cold-hardiness. O'Neill (1999) found that the timing of bud flush and bud set in Douglas-fir is strongly correlated (mean $|r_g|$=0.89) in sapling-age and older trees. Given that fall cold-hardiness is weakly associated with bud set (Aitken and Adams 1996; O'Neill 1999) there should be, at least, a weak association of spring bud flush with fall cold-hardiness. The small overlap of QTLs observed for these traits may represent a common set of genes that are involved in the expression of both traits.

Conclusion

We have detected several QTLs for fall and spring cold-hardiness in a three-generation outbred pedigree of coastal Douglas-fir, with slightly more QTLs being detected for spring cold-hardiness than for fall cold-hardiness. Individual QTLs explained a small percent of the total phenotypic variation, with the largest QTL explaining 9.8%. Many of the QTLs detected were additive in effect; the largest non-additive effect was detected on LG 7 for spring cold-hardiness in the needles. For spring cold-hardiness, several of the same QTLs were detected in all three tissue types measured, suggesting synchronization of de-acclimatization. However, for fall cold-hardiness, repeated expression of QTLs among tissue types was not detected, substantiating previous reports that there is little synchronization of hardening in the fall. Co-location of QTLs for both spring and fall cold-hardiness and QTLs for spring bud flush was observed.

Previous genetic studies on cold-hardiness have determined that: (1) spring cold-hardiness and fall cold-hardiness are weakly to moderately and negatively correlated, suggesting that the overlap of genes controlling these two traits is small; (2) correlations among cold-injury scores for different tissues in the spring are stronger than those estimated for the fall; and (3) spring bud flush is strongly correlated to spring cold-hardiness. Our QTL analyses suggest that: (1) there is little or no overlap of genes controlling cold-hardiness for spring and fall; (2) there is an overlap of genes controlling cold-hardiness among tissues in the spring but not in the fall; and (3) there is an overlap of genes regulating the expression of spring bud flush and both spring and fall cold-hardiness.

Our QTL estimations, based on a genetic linkage map, corroborates previous quantitative genetic analyses in that we find similar interrelationships among adaptive traits in Douglas-fir. However, the estimation of QTLs allows us to determine not only the location of genes involved in the timing and expression of these traits but also which genes may have a pleiotropic effect. This type of information will build a more-refined understanding of the genetic control of polygenic traits in forest trees.

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References

Aitken SN, Adams WT (1995) Screening for cold-hardiness in coastal Douglas-fir. In: Potts BM, Borralho NMG, Reid JB, Cromer RN, Tibbits WN, Raymond CA (eds) Eucalypt plantations: improving fibre yield and quality. Proc CRC/IUFRO Conf, 19–24 Geb. 1995, Hobart, Aust, CRC for Temperate Hardwood Forestry, Hobart, Aust, pp 321–324

Aitken SN, Adams WT (1996) Genetics of fall and winter cold-hardiness of coastal Douglas-fir in Oregon. Can J For Res 26:1828–1837

Aitken SN, Adams WT (1997) Spring cold-hardiness under strong genetic control in Oregon populations of coastal Douglas-fir. Can J For Res 27:1773–1780

Aitken SN, Hannerz M (2000) Genecology and gene resource management strategies for conifer cold hardiness. In: Bigras F, Colombo S (eds) Conifer cold hardiness. Kluwer Academic Press p. 33

Anekonda TS, Adams WT, Aitken SN (1998) Influence of second flushing on genetic assessment of cold-hardiness in coastal Douglas-fir [*Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco]. For Ecol Manag 111:119–126

Anekonda TS, Adams WT, Aitken SN (2000a) Cold-hardiness testing for Douglas-fir tree improvement programs: guidelines for a simple, robust and inexpensive method. Western J Appl For V15:129–136

Anekonda TS, Adams WT, Aitken SN, Neale DB, Jermstad KD, Wheeler NC (2000b) Genetics of cold-hardiness in a cloned full-sib family of coastal Douglas-fir. Can J For Res V30:837–840

Byrne M, Murrell JC, Owen JV, Williams ER, Moran GF (1997) Mapping of quantitative trait loci influencing frost tolerance in *Eucalyptus nitens*. Theor Appl Genet 95:975–979

Greer DH, Stanley CJ, Warrington IJ (1989) Photoperiod control of the initial phase of frost hardiness in *Pinus radiata*. Plant Cell Envir 12:661–668

Jermstad KD, Bassoni DL, Wheeler NC, Neale DB (1998) A sex-averaged genetic linkage map in coastal Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco var *'menziesii'*] based on RFLP and RAPD markers. Theor Appl Genet 97:762–770

Jermstad KD, Bassoni DL, Jech KS, Wheeler NC, Neale DB (2001) Mapping of quantitative trait loci controlling adaptive traits in coastal Douglas-fir. I. Spring bud flush. Theor Appl Genet 102:1142–1151

Johnsen O, Skroppa T, Junttila O, Daehlen OG (1996) Influence of the female flowering environment on autumn frost-hardiness of *Picea abies* progenies. Theor Appl Genet 92:797–802

Knott S, Neale DB, Sewell MM, Haley CS (1997) Multiple marker mapping quantitative trait loci in an outbred pedigree of loblolly pine. Theor Appl Genet 94:810–820

Neale DB, Jermstad KD, Sewell MM (1999) Progress towards detecting and verifying QTLs for wood property traits in loblolly pine and adaptive traits in Douglas-fir. In: Tree improvement: its contribution to sustainable development. Proc26th Meeting Canadian Tree Improvement Assoc, Part 1. August 18–21, 1997, Saint-Foy, Quebec

O'Neill GA (1999) Genetics of fall, winter and spring cold-hardiness in coastal Douglas-fir seedlings. PhD Dissertation, Oregon State University

Rehfeldt GE (1979) Variation in cold-hardiness among populations of *Pseudotsuga menziesii* var. *glauca*. Intermountain Forest and Range Experiment Station: USDA Forest Service Research Paper INT-233

Ritchie, GA (1993) Production of Douglas-fir rooted cuttings for reforestation by Weyerhaeuser Company. IPPS Proc 43:68–72

SAS Institute Inc (1989–1996) Release 6.12 edition. Cary, North Carolina

Schermann N, Adams WT, Aitken SN, Bastien J-Ch (1997) Genetic parameters of stem form traits in a 9 year-old coastal Douglas-fir progeny test in Washington. Silvae Genet 46:16–170

Timmis R, Flewelling J, Talber C (1994) Frost injury prediction model for Douglas-fir seedlings in the Pacific Northwest. Tree Physiol 14:855–869

White TL (1987) Drought tolerance of southwestern Oregon Douglas-fir. For Sci 33:283–293