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# Association Genetics of Coastal Douglas Fir (*Pseudotsuga menziesii* var. *menziesii*, Pinaceae). I. Cold-Hardiness Related Traits

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ABSTRACT

Adaptation to cold is one of the greatest challenges to forest trees. This process is highly synchronized with environmental cues relating to photoperiod and temperature. Here, we use a candidate gene-based approach to search for genetic associations between 384 single-nucleotide polymorphism (SNP) markers from 117 candidate genes and 21 cold-hardiness related traits. A general linear model approach, including population structure estimates as covariates, was implemented for each marker–trait pair. We discovered 30 highly significant genetic associations [false discovery rate (FDR) $Q < 0.10$] across 12 candidate genes and 10 of the 21 traits. We also detected a set of 7 markers that had elevated levels of differentiation between sampling sites situated across the Cascade crest in northeastern Washington. Marker effects were small ($r^2 < 0.05$) and within the range of those published previously for forest trees. The derived SNP allele, as measured by a comparison to a recently diverged sister species, typically affected the phenotype in a way consistent with cold hardiness. The majority of markers were characterized as having largely nonadditive modes of gene action, especially underdominance in the case of cold-tolerance related phenotypes. We place these results in the context of trade-offs between the abilities to grow longer and to avoid fall cold damage, as well as putative epigenetic effects. These associations provide insight into the genetic components of complex traits in coastal Douglas fir, as well as highlight the need for landscape genetic approaches to the detection of adaptive genetic diversity.

A fundamental goal of molecular population and quantitative genetics is to discover polymorphisms that underlie adaptive phenotypic traits. Elucidation of the genetic components for ecologically relevant traits within natural populations has been slow, due mostly to the disconnect between organisms with detailed genomic resources and those that have phenotypes with ecological relevance (Stinchcombe and Hoekstra 2008). Rapid advances and applications of high-throughput marker technologies are beginning to amend this disconnect for forest trees. Several applications of association mapping approaches using functional marker data have been fruitful in identifying putatively causal single-nucleotide polymorphisms (SNPs) for an array of adaptive phenotypes across different forest tree species. The importance of these associations is clear, with putative applications ranging from marker-assisted breeding to gene conservation in the face of climate change (Walther *et al.* 2002; Aitken *et al.* 2008).

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Adaptation to cold is one of the greatest challenges to forest trees and is highly synchronized with environmental cues, primarily photoperiod and temperature (Saxe *et al.* 2001; Howe *et al.* 2003). The annual growth cycle of temperate forest trees involves a trade-off between the timing of initiation and cessation of growth that takes full advantage of favorable climatic conditions, while avoiding cold damage from late frosts in the spring and early frosts in the fall. Timing of bud flush is predominantly influenced by temperature following adequate chilling, while bud set is influenced by photoperiod (short days), as well as temperature, soil moisture, nutrition, and light quality (Sakai and Larcher 1987; Howe *et al.* 2003). The first stage of cold hardiness is also induced by short days, while low temperatures induce the second stage (Weiser 1970; Sakai and Larcher 1987).

Here, we take an association genetic approach to the dissection of cold-hardiness related traits within natural populations of coastal Douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco var. *menziesii*]. The range of this species extends from the Pacific Coast of North America to the eastern slope of the Rocky Mountains, with trees from the Pacific Coast classified as *P. menziesii* var. *menziesii*

and those from the Rocky Mountains classified as *P. menziesii* var. *glauca* (Bessin.) Franco. The success of Douglas fir across this highly heterogeneous landscape is due largely to its ability to maximize growth during favorable climatic conditions, balanced with tolerance to low temperatures (Rehfeldt 1989; St. Clair *et al.* 2005; St. Clair 2006).

Genetic variation for cold hardiness in coastal Douglas fir is well documented among geographic sources and among families within sources (Campbell and Sorensen 1973; White 1987; Loopstra and Adams 1989; Aitken and Adams 1996, 1997; O'Neill *et al.* 2001; St. Clair 2006). Most of these traits are also heritable, with $h^2$ values ranging from 0.10 to 0.85. Population differences in cold adaptation across the range of Douglas fir are strongly influenced by geographic and climatic variables (Howe *et al.* 2003). Differences in cold season temperature and associated geographic variables (*e.g.*, latitude, elevation, and distance from the ocean) are important selective forces driving local adaptation of populations (St. Clair *et al.* 2005). For example, population differentiation at quantitative traits ($Q_{ST}$) related to fall cold hardiness is eightfold greater than differentiation at anonymous and presumably neutral markers ($F_{ST}$), suggesting the action of natural selection acting upon these traits (St. Clair 2006). The genes underlying cold hardiness, however, have remained elusive, despite numerous efforts to map quantitative trait loci (QTL) (Jermstad *et al.* 2001a,b, 2003) and to analyze patterns of collocation between QTL and candidate genes (Wheeler *et al.* 2005).

Expression studies support the hypothesis that similar types of genes to those identified in Arabidopsis are involved with cold adaptation in conifers (Guy *et al.* 1985; Thomashow 1999; Fowler and Thomashow 2002; Sekai *et al.* 2002; Lee *et al.* 2005; Yakovlev *et al.* 2006; Holliday *et al.* 2008). Population genetic investigations into patterns of diversity and divergence at candidate genes for cold adaptation, as well as a suite of other adaptive phenotypes, however, often find few loci consistent with the action of natural selection (Brown *et al.* 2004; Krutovsky and Neale 2005; González-Martínez *et al.* 2006; Heuertz *et al.* 2006; Ingvarsson *et al.* 2006; Hall *et al.* 2007; Pyhäjärvi *et al.* 2007; Eveno *et al.* 2008) (reviewed by Neale 2007; Savolainen and Pyhäjärvi 2007; Neale and Ingvarsson 2008). Even the low power of the methods employed in these investigations (Zhai *et al.* 2009) and the theoretical expectations that selected loci may be unable to be detected using outlier approaches (Le Corre and Kremer 2003) are unlikely to account for the paucity of results. Larger sets of candidate genes are crucial, therefore, for the continued investigation and identification of major portions of the adaptive genetic diversity in forest trees.

Similar patterns have been found in association genetic analyses, where only a small number of markers all of small effect are detected (Neale and Savolainen 2004; Thumma *et al.* 2005; González-Martínez *et al.* 2007, 2008; Ingvarsson *et al.* 2008) (reviewed by Neale 2007; Grattapaglia and Kirst 2008; Grattapaglia *et al.* 2009). Much of this work has focused on point mutations within coding regions, thus ignoring regulatory regions affecting gene expression. Seminal work has illuminated the possibility that many of the adaptive responses by forest trees to their environments, however, may stem from epigenetic effects (Johnsen *et al.* 1996; Hänninen *et al.* 2001; Saxe *et al.* 2001; Johnsen *et al.* 2005a,b; Webber *et al.* 2005; Kvaalen and Johnsen 2008). The prevalence of such effects modifies the expectation of the quantity, type, and effect size of genes involved with adaptation by forest trees.

The segregation of adaptive genetic diversity by coastal Douglas fir along environmental gradients is clearly established. Surveys of molecular diversity and divergence across 139 candidate genes have documented a set of those genes that deviate from the standard neutral model (Krutovsky and Neale 2005; Eckert *et al.* 2009b). These are prime candidates for the further dissection of cold-hardiness related traits using association mapping (*cf.* Wright and Gaut 2005). Here, we aim to bridge the gap between molecular population and quantitative genetics, using an association mapping approach. Our primary goal is to identify single-marker associations with 21 cold-hardiness traits. In doing so, we highlight the need for future investigations into landscape approaches to the description of adaptive genetic diversity, as well as studies of epigenetic effects in coastal Douglas fir.

## MATERIALS AND METHODS

**Association population and phenotypic data:** *Association population:* The association population consisted of 700 of the 1338 unrelated families that were assessed in the genecology study of St. Clair *et al.* (2005). They represent an extensive rangewide sample covering 6.8° of latitude, 4.1° of longitude, and a diversity of environmental conditions (Figure 1; supporting information, Table S1). Wind-pollinated seed was collected from trees that originated from naturally regenerated stands throughout the range of Douglas fir in western Oregon and Washington. Twenty progeny were grown in raised nursery beds that were located in Corvallis, Oregon. Families were randomly assigned to five-tree row plots in each of the four raised beds, with each bed treated as a block. The term family is used to refer to source trees (*i.e.*, mothers) because the phenotypic values we use are breeding values, and this is the terminology used in the original studies in which the phenotypes were measured (*cf.* St. Clair *et al.* 2005; St. Clair 2006).

*Phenotypes:* Seedlings were grown for 2 years, during which they were measured for 21 traits related to cold injury, emergence, bud phenology, growth, and resource partitioning (Table 1). The data for cold-tolerance traits were obtained from St. Clair (2006). Emergence was determined following procedures described by Campbell and Sorensen (1979). Height and bud set were measured at the end of the first

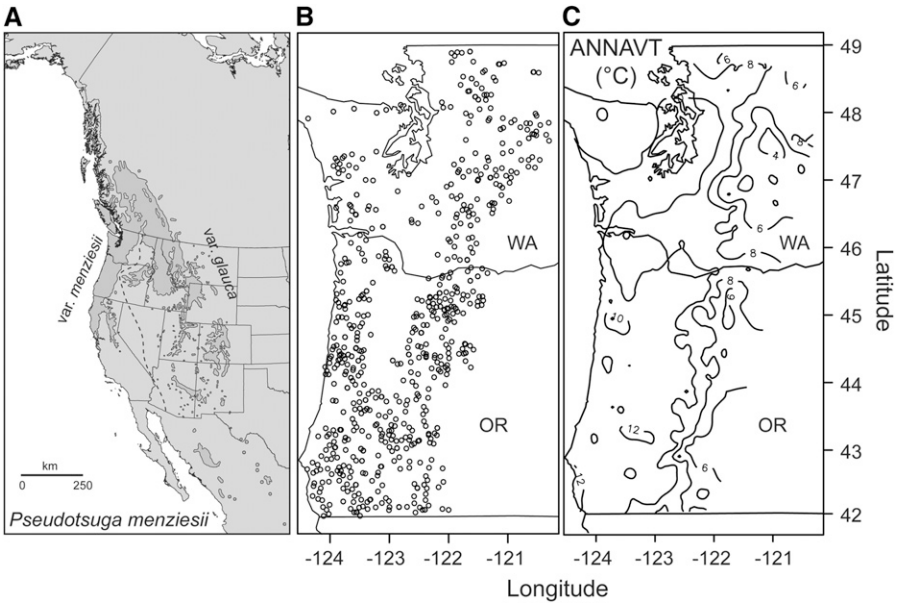


FIGURE 1.—Descriptive information about the distribution, sampling localities, and climate across the range of coastal Douglas fir. (A) Range map for coastal Douglas fir. (B) Sample locations for coastal Douglas fir across Oregon and Washington. Each point denotes a single tree ($n = 700$) that was sampled, and since the phenotypes represent breeding values, these samples are referred to as families. (C) The annual average temperature (ANNAVT) gradient across the sample localities. Contours are isotherms, ranging from 6° to 12°.

growing season, and bud burst was measured at the beginning of the second growing season. Bud set was also measured at the end of the second growing season. Samples were frozen in a programmable freezer and fall cold hardiness was assessed on needle, stem, and bud tissues after the second growing season following the methods of AITKEN and ADAMS (1996). Whole seedlings, including roots, were then harvested and were measured for stem diameter, height from root collar to terminal bud, height to bud scar resulting from second flushing, and length of the longest root. Dry weights of roots and shoots were determined after drying the seedlings at 80° for 24 hr. Values for each phenotypic trait were calculated as the grand mean of family plot means. Year-to-year environmental variation was removed by standardizing the plot mean data, so that the means and standard deviations of control plots were equal across years.

Individual phenotypic traits were highly correlated (Pearson's $r$: −0.71–0.94). To account for these correlations, multivariate traits were constructed with a principal components analysis (PCA), using PROC PRINCOMP with the correlation matrix in the SAS software (SAS system for Windows, version 9.1, Copyright 2002; SAS Institute, Cary, NC). We retained all components with eigenvalues greater than one. In total these components accounted for 80.0% of the variance. Factor loadings for each component are located in Table S2.

**SNP genotyping:** *DNA isolation:* Total genomic DNA was isolated using the DNeasy plant mini kit (QIAGEN, Valencia, CA) and quantified using the PicoGreen assay (Invitrogen, Carlsbad, CA). For each maternal tree, haploid megagametophyte tissue excised from 10 seeds was combined and ground under liquid nitrogen. Inferring the diploid maternal genotype from haploid tissues can result in a bias against detection of heterozygotes. Using 10 megagametophytes, however, results in only a ~0.2% expected probability of this type of error (MORRIS and SPIETH 1978). All DNA extractions were carried out at the U.S. Department of Agriculture Forest Service, National Forest Gel Electrophoresis Laboratory at the Institute of Forest Genetics (Placerville, CA).

*Candidate gene selection:* Candidate genes with a putative role in conferring tolerance to cold temperatures were selected according to three criteria: (i) genes found to collocate with QTL for cold hardiness in Douglas fir, (ii) genes with physiological roles in cold tolerance response, and (iii) genes showing differential expression in microarray studies of Arabidopsis. A full description of the candidate gene selection process can be found elsewhere (KRUTOVSKY and NEALE 2005; ECKERT *et al.* 2009b). In brief, we used the 939 genes identified by LEE *et al.* (2005) as cold regulated in Arabidopsis to mine Douglas fir expressed sequence tag (EST) libraries using standard BLAST tools. Putative homologs were sequence validated prior to construction of the final candidate gene list.

*SNP discovery and selection:* The discovery of SNPs was conducted previously by direct sequencing of haploid megagametophyte DNA samples in a diversity panel of 23–32 trees for 18 (KRUTOVSKY and NEALE 2005) and 121 (ECKERT *et al.* 2009b) cold-hardiness and wood-related candidate genes. From those sets (400 SNPs from KRUTOVSKY and NEALE 2005; 933 SNPs from ECKERT *et al.* 2009b) we selected 384 SNPs from 117 genes with which to construct a GoldenGate genotyping assay (Illumina, San Diego). This platform has been shown previously to work well for conifer genomes (PAVY *et al.* 2008; ECKERT *et al.* 2009a). Selection of SNPs was based on four criteria: (i) gene function, (ii) SNP annotation, (iii) Illumina designability score, and (iv) minor allele frequency. One to 12 SNPs per gene were selected to capture most of the haplotypic variation within candidate genes (Table S3).

*SNP genotyping:* Genotyping was carried out using the Illumina GoldenGate SNP genotyping platform (LANDEGREN *et al.* 1988; OLIPHANT *et al.* 2002; FAN *et al.* 2003). In brief, this assay involves generating hundreds of templates with specific target and address sequences, using allele-specific extension followed by ligation and amplification with universal primers. Fluorescent products are hybridized to precoded beads on an array matrix from which the signal intensities are subsequently determined using the BeadArray Reader (Illumina). This is followed by quantification and matching of those intensities to specific alleles using BeadStudio ver. 3.1.14 (Illumina). Manual adjustments to genotypic clusters were made when necessary. For inclusion of SNPs into the final data set, we used conservative thresholds of 0.35 and 0.85 for the GenCall$_{50}$ ($GC_{50}$) and call rate (CR) indexes, respectively. These are common quality metrics with which to evaluate the successfulness of Illumina genotyping data (*cf.* PAVY *et al.* 2008; ECKERT *et al.* 2009a) and represent the reliability of samples to be clustered into genotypic categories ($GC_{50}$) and the fraction of the 700 samples that had a genotype called for a given SNP (CR). Genotyping was conducted at the DNA Technologies Core Facility located at the University of

**TABLE 1**

**Description of measured traits listed by phenotypic categories**

| Trait | Abbreviation | Description | Unit |
|---|---|---|---|
| Emergence | | | |
| Rate of emergence | EMEAN | Cumulative no. of seedlings that emerged in a plot | Probits $d^{-1}$ |
| Standard deviation | EMSTD[a] | Standard deviation of the rate of emergence | Probits $d^{-1}$ |
| Growth and resource partitioning | | | |
| Stem diameter | DIAM | At 1 cm above root collar after 2 yr | mm |
| Propensity to 2nd flush | FLUSH | Proportion of 2-yr seedlings with lammas growth of terminal leader | Proportion |
| Length of 2nd flush | FLUSHLG | Distance from visible bud scar to base of terminal bud | cm |
| Height | HT1 | From root collar to base of terminal bud after 1 yr | cm |
| Height | HT2[a] | From root collar to base of terminal bud after 2 yr | cm |
| Height increment | HTINC | HT2–HT1 | cm |
| Root length | RTLG | From root collar to tip of longest root | mm |
| Root-to-shoot ratio | RTSH | Ratio of dry weights after 2 yr | g $g^{-1}$ |
| Root weight | RTWT | Root dry weight after 2 yr | g |
| Shoot weight | SHWT | Shoot dry weight after 2 yr | g |
| Taper | TAPER | DIAM/HT2 | mm $cm^{-1}$ |
| Total weight | TOTWT[a] | Sum of shoot and root weights | g |
| Phenology and cold tolerance | | | |
| Bud burst | BB2 | First green needles from terminal bud | Days since Jan. 1 |
| Bud set | BS1 | First visible terminal bud scales at end of first growing season | Days since Jan. 1 |
| Bud set | BS2 | First visible terminal bud scales at end of second growing season | Days since Jan. 1 |
| Bud cold injury | Budcold | Percentage of cold injury | % |
| Needle cold injury | Ndlcold | Percentage of cold injury | % |
| Stem cold injury | Stmcold | Percentage of cold injury | % |
| Other | | | |
| Seed weight | SDWT[b] | Weight per 100 seeds | g |

Loadings of derived traits from PCA are listed in Table S2.
[a] Variables not included in PCA since the trait is a linear combination of other traits.
[b] Nonseedling trait, not included in PCA.

California, Davis (http://www.genomecenter.ucdavis.edu). Primer sequences used for SNP discovery and Illumina genotyping are available in File S1 and File S2).

**Tests for association:** *Population structure:* Population structure is the leading cause of false positives in genetic association studies. Populations of coastal Douglas fir are not differentiated strongly from one another using allozymes (Li and Adams 1989), RAPDs (Aagaard *et al.* 1998), or chloroplast and nuclear microsatellites (Viard *et al.* 2001; Krutovsky *et al.* 2009). The average level of population differentiation ($G_{ST}$) was ~0.02–0.07 in all studies, with Li and Adams (1989) noting weak to moderate ($r < 0.30$) isolation-by-distance effects in the coastal populations. Low levels of population structure can be observed in widespread species when populations as defined *a priori* are not meaningful biologically (Waples and Gaggiotti 2006). Applications of the Bayesian clustering algorithm in the program STRUCTURE (*cf.* Falush *et al.* 2003) produce results that concur with the observed low values of $G_{ST}$, with most individuals being assigned equally well to all of the assumed clusters ($K$) across values of $K$ ranging from 2 to 18 (Krutovsky *et al.* 2009). For association analyses, we utilized a $Q$-matrix defined by 15 clusters, because this was the smallest value of $K$ producing a large (*i.e.*, >100 log units) change in the log probability of the data. This matrix was estimated using 25 isozymes and six nuclear microsatellite markers for the same families as those presented here.

Common garden studies indicate that a set of 57 families sampled east of the Cascade crest in northeastern Washington resembled the interior variety more so than the coastal variety for cold tolerance, phenology, and growth phenotypes (St. Clair *et al.* 2005). Genetic differentiation of these families was assessed using hierarchical analysis of molecular variance (AMOVA) with 25 allozyme markers (data from Krutovsky *et al.* 2009). We defined populations according to 20 ecological regions and then placed those populations into groups corresponding to populations located to the west or the east of the Cascade crest in northeastern Washington (Figure S1). Confidence intervals (95% C.I.'s) for global fixation indexes corresponding to $F_{CT}$ (between groups), $F_{SC}$ (among populations within groups), and $F_{IS}$ (within individuals) across loci were determined by bootstrapping ($n = 20{,}000$ replicates). Global fixation indexes were obtained by summing variance components across loci. All analyses were conducted in Arlequin ver. 3.11 (Excoffier *et al.* 2005). These analyses were used to investigate further population structure putatively not captured by patterns in the $Q$-matrix obtained from Krutovsky *et al.* (2009).

*Statistical models:* Single-marker models were conducted for all SNP–trait combinations. We preferred single-marker relative to haplotype-based tests due to their simplicity, as well as to their similar statistical power to that of haplotype-based tests (Long and Langley 1999). A general linear model (GLM)

was fitted to each trait–SNP combination (*cf.* Yu *et al.* 2006), with SNP markers as fixed effects and elements of the *Q*-matrix as covariates. We removed the first cluster in the *Q*-matrix because if included it would make an unnecessary linear dependence among the covariates when we performed *F*-tests of the covariates. All GLM analyses were conducted using Tassel ver. 2.0.1 (released April, 2007). The positive false discovery rate (FDR) method was used to correct for multiple testing (Storey 2003). All the necessary data to perform these analyses are available in File S1, File S2, File S3, File S4, and File S5).

*Modes of inheritance and LD:* The prevalence of nonadditive effects was quantified using the ratio of dominance ($d$) to additive ($a$) effects. Partial or complete dominance was defined as values in the range of $0.50 < |d/a| < 1.25$, while additive effects were defined as values in the range $-0.50 \leq d/a \leq 0.50$. Values of $|d/a| > 1.25$ were equated with over- or underdominance. We also investigated patterns of linkage disequilibrium (LD) among SNPs that were associated significantly with the same trait, using the maximum-likelihood approach implemented within the GENETICS package available in R (Warnes and Leisch 2006). We quantified patterns of LD using the squared allelic correlation coefficient ($r^2$) and tested the significance of the inferred level of disequilibrium using Fisher's exact tests with a Bonferroni correction to account for multiple testing.

## RESULTS

**Data summary:** The 384 SNPs chosen for genotyping using the Illumina GoldenGate platform represent 117 unique candidate genes with 1–12 SNPs per gene (Table S3). Of the 384 SNPs, 228 (59%) yielded data consistent with our quality thresholds. The median $GC_{50}$ score across all usable SNPs was 0.742, with the average CR being 94%. The majority of the 228 successfully genotyped SNPs were silent, with nonsynonymous SNPs accounting for 25% of the total. This did not deviate greatly from the original fraction in the full 384-SNP set (28%).

**Population structure:** Strong patterns of population stratification were not apparent in the results obtained from Krutovsky *et al.* (2009). Most individuals were assigned equally well to one of the $K$ clusters (*i.e.*, $Q \sim 1/K$). Latitude and longitude were largely uncorrelated to the *Q*-values for each of the 15 clusters, but patterns were apparent. Four clusters illustrated a correlation of *Q*-values to geography, with one of the 15 clusters corresponding to the 57 families located east of the Cascade crest in northeastern Washington (Figure S2). Differentiation across 25 allozyme markers for these families is moderate (global $F_{CT} = 0.035$; 95% C.I., 0.015–0.082) and accounts for >90% of the differentiation among populations. Inbreeding within populations, however, was not significant ($F_{IS} = 0.026$; 95% C.I., −0.005–0.055). The trait means for these families also differ significantly for 23 of the 25 traits (Table S4). We focus on association analyses that have these 57 families removed and then compare the results to those obtained when they are included.

**Summary of significant associations:** A total of 5700 (228 SNPs × 25 traits) association tests were performed. Of these, 455 were significant at the nominal threshold of $P = 0.05$. Multiple test corrections using the FDR method reduced this number to 30 at a significance threshold of $Q = 0.10$. Of these, four marker–trait pairs remain significant after a conservative Bonferroni correction (Table 2). The number of significant associations varied across traits, ranging from 0 to 6. The 30 significant associations represent 15 unique SNPs from 12 candidate genes that affect 10 different traits. We discuss these associations in further detail below.

**Individual phenotypic traits:** *Growth and resource partitioning traits:* Twelve of the 21 traits were related to growth and resource partitioning (Table 1). These traits had a total of four significant marker–trait associations located within four unique candidate genes. One of these associations, the effect of marker *ES421311.1*-369 on root length (RTLG), survives a Bonferroni correction. These markers explain a small portion of the phenotypic variance in our sample, with effects ranging from 1.9 to 3.6%.

*Phenology and cold-tolerance traits:* Six of the 21 traits were related to phenology and cold tolerance (Table 1). These traits had a total of 18 significant associations representing 11 unique SNPs located within 10 different candidate genes. One of these 18 associations survives a Bonferroni correction (Table 2). The majority of these SNPs illustrated patterns of gene action consistent with nonadditive effects (Table 3). For example, heterozygotes for the *CN637339.1*-367 marker set bud 3 days later on average than either homozygote class (274.3 for A/A, 277.7 for A/G, 275.1 for G/G). This marker is also associated with cold damage to stems and illustrated a similar mode of gene action, with heterozygotes having 0.35% more cold damage on average (2.1 for A/A, 2.4 for A/G, 1.9 for G/G). These 2 traits, however, are correlated with one another. The G allele at this marker is the derived state and causes an isoleucine (Ile) → valine (Val) amino acid substitution. Interestingly, all 5 additional marker–trait associations for cold damage to the stem phenotype illustrate a similar pattern, with heterozygotes having significantly more damage.

Polymorphisms located within different candidate genes that were associated to the same trait were largely in linkage equilibrium. Departures from this pattern, however, were apparent. Significant pairwise estimates of LD were documented between markers located in the *CN637306.1*, *f3h2*, *Pm_CL234Contig1*-156, and *CN638489.1* candidate gene loci. These three genes have SNPs associated significantly with cold damage to the stems, with 2.5–3.4% of the phenotypic variance being explained by each marker. The departures from linkage equilibrium are small ($r^2 < 0.20$, $P < 0.0001$), yet define a set of candidate genes whose products are a transcription factor (*CN63730.1*), a rab GTPase (*Pm_CL234Contig1*), a cell wall architecture protein (α-expansin), and a flavanoid pathway protein (*f3h2*). Marker *CN63730.1*-381, however, deviates significantly

**TABLE 2**

**List of significant marker–trait pairs after a correction for multiple testing (FDR $Q \leq 0.10$), using the 643 families located west of the Cascade crest**

| Trait | Locus | Gene product | SNP[a] | AS[b] | $n$ | $F$ | $r^2$ | $P$ | $Q$ |
|---|---|---|---|---|---|---|---|---|---|
| Emergence | | | | | | | | | |
| EMEAN | *60s RPL31a*-418 | 60s ribosomal protein L31a | [A/G][c] | — | 570 | 9.501 | 0.032 | 0.0001 | 0.0372 |
| EMEAN | *CN639236.1*-518 | Guanine nucleotide-binding protein | **[A/G]**[d] | A | 617 | 8.092 | 0.025 | 0.0003 | 0.0765 |
| Growth and resource partitioning | | | | | | | | | |
| RTLG | *ES421311.1*-369 | Hypothetical protein | [A/G][c] | G | 642 | 23.841 | 0.036 | $1.3 \times 10^{-6}$ | 0.0038 |
| RTSH | *Pm_CL783Contig1*-212 | SOUL heme-binding family protein | [A/G][e] | C | 641 | 13.696 | 0.021 | 0.0002 | 0.0573 |
| RTSH | *4CL1*-363 | 4-coumarate:CoA ligase 1 | [A/G][e] | — | 643 | 12.936 | 0.019 | 0.0003 | 0.0765 |
| TAPER | *LEA-EMB11*-263 | Late embryogenesis abundant protein | [A/C][c] | — | 614 | 13.832 | 0.022 | 0.0002 | 0.0561 |
| Phenology and cold tolerance | | | | | | | | | |
| BB2 | *LEA-EMB11*-263 | Late embryogenesis abundant protein | [A/C][c] | — | 614 | 16.748 | 0.025 | $4.9 \times 10^{-5}$ | 0.0342 |
| BB2 | *60s RPL31a*-295 | 60s ribosomal protein L31a | [A/G][d] | — | 641 | 12.834 | 0.019 | 0.0004 | 0.0765 |
| BB2 | *60s RPL31a*-418 | 60s ribosomal protein L31a | [A/G][c] | — | 570 | 7.607 | 0.024 | 0.0006 | 0.0913 |
| BS1 | *CN637339.1*-337 | Hypothetical protein | [A/G][e] | A | 599 | 9.029 | 0.029 | 0.0001 | 0.0429 |
| BS1 | *Pm_CL783Contig1*-212 | SOUL heme-binding family protein | [A/G][e] | C | 641 | 12.485 | 0.019 | 0.0004 | 0.0841 |
| BS2 | *60s RPL31a*-418 | 60S ribosomal protein L31a | [A/G][c] | — | 570 | 7.752 | 0.026 | 0.0005 | 0.0841 |
| Budcold | *4CL1*-520 | 4-coumarate:CoA ligase 1 | [A/G][e] | — | 641 | 9.118 | 0.028 | 0.0001 | 0.0427 |
| Budcold | *CN638489.1*-116 | α-expansin | [A/G][d] | C | 628 | 7.607 | 0.024 | 0.0005 | 0.0913 |
| Ndlcold | *sSPcDFD040B03103*-274 | MADS-box transcription factor | [A/G][d] | C | 641 | 20.392 | 0.03 | $7.5 \times 10^{-6}$ | 0.0071 |
| Ndlcold | *CN637306.1*-381 | MYB-like transcription factor | **[A/G]**[d] | T | 642 | 8.563 | 0.026 | 0.0002 | 0.0561 |
| Ndlcold | *4CL1*-520 | 4-coumarate:CoA ligase 1 | [A/G][e] | — | 641 | 7.875 | 0.024 | 0.0004 | 0.0841 |
| Ndlcold | *f3h2*-54 | Flavanone-3-hydroxylase | **[A/C]**[c] | — | 642 | 7.749 | 0.023 | 0.0005 | 0.0841 |
| Stmcold | *CN637306.1*-381 | MYB-like transcription factor | **[A/G]**[d] | T | 642 | 11.485 | 0.034 | $1.3 \times 10^{-5}$ | 0.0101 |
| Stmcold | *f3h2*-54 | Flavanone-3-hydroxylase | **[A/C]**[e] | — | 642 | 9.427 | 0.028 | 0.0001 | 0.0372 |
| Stmcold | *Pm_CL234Contig1*-156 | Rab GTPase | [A/T][e] | T | 594 | 8.791 | 0.029 | 0.0002 | 0.0513 |
| Stmcold | *CN637339.1*-337 | Hypothetical protein | [A/G][e] | A | 599 | 8.593 | 0.028 | 0.0002 | 0.0561 |
| Stmcold | *CN638489.1*-116 | α-expansin | [A/G][d] | C | 628 | 8.036 | 0.025 | 0.0004 | 0.0765 |
| Stmcold | *sSPcDFD040B03103*-274 | MADS-box transcription factor | [A/G][d] | C | 641 | 12.34 | 0.019 | 0.0005 | 0.0841 |
| Multivariate traits | | | | | | | | | |
| Prin1 | *Pm_CL783Contig1*-212 | SOUL heme-binding family protein | [A/G][e] | C | 641 | 15.878 | 0.024 | 0.0001 | 0.0372 |
| Prin1 | *sSPcDFD040B03103*-274 | MADS-box transcription factor | [A/G][d] | C | 641 | 14.843 | 0.023 | 0.0001 | 0.0427 |
| Prin2 | *60s RPL31a*-418 | 60s ribosomal protein L31a | [A/G][c] | — | 570 | 12.648 | 0.043 | $4.3 \times 10^{-6}$ | 0.0048 |
| Prin2 | *60s RPL31a*-55 | 60s ribosomal protein L31a | [A/G][d] | — | 571 | 9.836 | 0.033 | 0.0001 | 0.0372 |
| Prin3 | *LEA-EMB11*-263 | Late embryogenesis abundant protein | [A/C][c] | — | 614 | 22.475 | 0.034 | $2.7 \times 10^{-6}$ | 0.0038 |
| Prin3 | *4CL1*-520 | 4-coumarate:CoA ligase 1 | [A/G][e] | — | 641 | 9.512 | 0.028 | 0.0001 | 0.0372 |

[a] SNPs in boldface type were not consistent with Hardy–Weinberg equilibrium (HWE) expectations, as tested using Fisher's exact tests, at a Bonferroni-corrected significance threshold of $P = 0.00022$ (*i.e.*, 0.05/228).

[b] The ancestral state as determined by comparison to a single sequence of bigcone Douglas fir (*Pseudotsuga macrocarpa*). Dashes indicate that an outgroup sequence was unavailable.

[c] Noncoding polymorphism.

[d] Synonymous polymorphism.

[e] Nonsynonymous polymorphism.

**TABLE 3**

**List of marker effects for significant marker–trait pairs using the 643 families located west of the Cascade crest**

| Trait | Locus[a] | Gene product | $2a$[b] | $d$[c] | $d/a$ | $2a/\sigma_p$[d] | Frequency[e] | $\alpha$[f] |
|---|---|---|---|---|---|---|---|---|
| Emergence | | | | | | | | |
| EMEAN | *60s RPL31a*-418 | 60s ribosomal protein L31a | 0.002 | 0.001 | 1.01 | 0.69 | 0.24 (G) | **−0.0004** |
| Phenology and cold tolerance | | | | | | | | |
| BB2 | *60s RPL31a*-418 | 60s ribosomal protein L31a | 3.26 | −1.21 | −0.73 | 0.74 | 0.24 (G) | **0.6916** |
| BS1 | *CN637339.1*-337 | Hypothetical protein | 0.73 | 2.80 | 7.68 | 0.10 | 0.21 (G) | 0.9737 |
| BS2 | *60s RPL31a*-418 | 60s ribosomal protein L31a | 7.36 | −1.54 | −0.42 | 0.62 | 0.24 (G) | **2.0844** |
| Budcold | *4CL1*-520 | 4-coumarate:CoA ligase 1 | 1.10 | −0.04 | −0.07 | 0.85 | 0.36 (G) | **−0.1740** |
| Budcold | *CN638489.1*-116 | α-expansin | 0.39 | 0.30 | 1.54 | 0.30 | 0.49 (A) | −0.0900 |
| Ndlcold | *4CL1*-520 | 4-coumarate:CoA ligase 1 | 1.78 | −0.11 | −0.12 | 0.85 | 0.36 (G) | **−0.2854** |
| Stmcold | *Pm_CL234Contig1*-156 | rab GTPase | 0.50 | 0.24 | 0.96 | 0.36 | 0.76 (A) | −0.0874 |
| Stmcold | *CN637339.1*-337 | Hypothetical protein | 0.31 | 0.35 | 2.23 | 0.22 | 0.21 (G) | 0.2791 |
| Stmcold | *CN638489.1*-116 | α-Expansin | 0.45 | 0.34 | 1.49 | 0.32 | 0.49 (A) | −0.1044 |
| Multivariate traits | | | | | | | | |
| Prin2 | *60s RPL31a*-418 | 60s ribosomal protein L31a | 1.36 | 0.47 | 0.70 | 0.83 | 0.24 (G) | **0.0251** |
| Prin2 | *60s RPL31a*-55 | 60s ribosomal protein L31a | 1.03 | 0.32 | 0.62 | 0.63 | 0.35 (A) | **0.0895** |
| Prin3 | *4CL1*-520 | 4-coumarate:CoA ligase 1 | 0.72 | 0.09 | 0.23 | 0.51 | 0.36 (G) | **−0.2366** |

[a] Markers with only two observed genotypic classes or those that deviated significantly from HWE expectations are not included.

[b] Calculated as the difference between the phenotypic means observed within each homozygous class ($2a = |G_{BB} - G_{bb}|$, where $G_{ij}$ is the trait mean in the $ij$th genotypic class).

[c] Calculated as the difference between the phenotypic mean observed within the heterozygous class and the average phenotypic mean across both homozygous classes [$d = G_{Bb} - 0.5(G_{BB} + G_{bb})$, where $G_{ij}$ is the trait mean in the $ij$th genotypic class].

[d] $\sigma_p$, standard deviation for the phenotypic trait under consideration. Prior to calculating this measure, the observed distributions of the trait values were tested for normality using Kolmogorov–Smirnov tests and a Bonferroni-corrected significance threshold of $P = 0.002$ (*i.e.*, 0.05/25).

[e] Allele frequency of either the derived or the minor allele. SNP alleles corresponding to the frequency listed are given in parentheses.

[f] The additive effect was calculated as $\alpha = p_B(G_{BB}) + p_b(G_{Bb}) - G$, where $G$ is the overall trait mean, $G_{ij}$ is the trait mean in the $ij$ th genotypic class, and $p_i$ is the frequency of the $i$th marker allele. These values were always calculated with respect to the derived allele. When this was unknown, the values listed are for the minor allele. Values calculated in the latter manner are in boldface type.

from Hardy–Weinberg equilibrium (HWE) expectations ($P < 0.0001$).

*Emergence:* Two of the 21 traits were related to emergence. These traits had a total of two significant associations representing two unique candidate genes. Both markers explained ~3% of the phenotypic variance. Mean values of emergence (EMEAN) across genotypic classes at marker *60s RPL31a*-418 indicate that the G allele is dominant to the A allele (0.0435 for A/A, 0.0459 for A/G, and 0.0458 for G/G). A similar analysis for the *CN639236.1*-518 locus was not performed, because the G/G genotype was not observed in our sample.

**Multivariate phenotypic traits:** Marker–trait associations with principal components mirrored those detected for individual traits. The first principal component accounted for a large fraction of the phenotypic variance (44.8%). This component was primarily composed of growth traits, with phenology and cold-tolerance traits also strongly affecting its composition (Table S2). Two SNPs located within two different candidate genes were associated significantly with this component. Both loci explained a small fraction of the phenotypic variance ($r^2 < 0.025$) and were associated primarily with individual traits related to phenology and cold tolerance. The second principal component was related largely to cold damage traits. Two markers located within the *60s RPL31a* locus were associated significantly with this component (Table 2). These markers were also in significant LD with one another and explained similar fractions of the phenotypic variance (Figure 2). The average values of this component across genotypic classes for each SNP are suggestive of partial dominance, with the A allele at the *60s RPL31a*-55 locus and the G allele at the *60s RPL31a*-418 locus being partially dominant (Figure 2). These are also the alleles that are associated

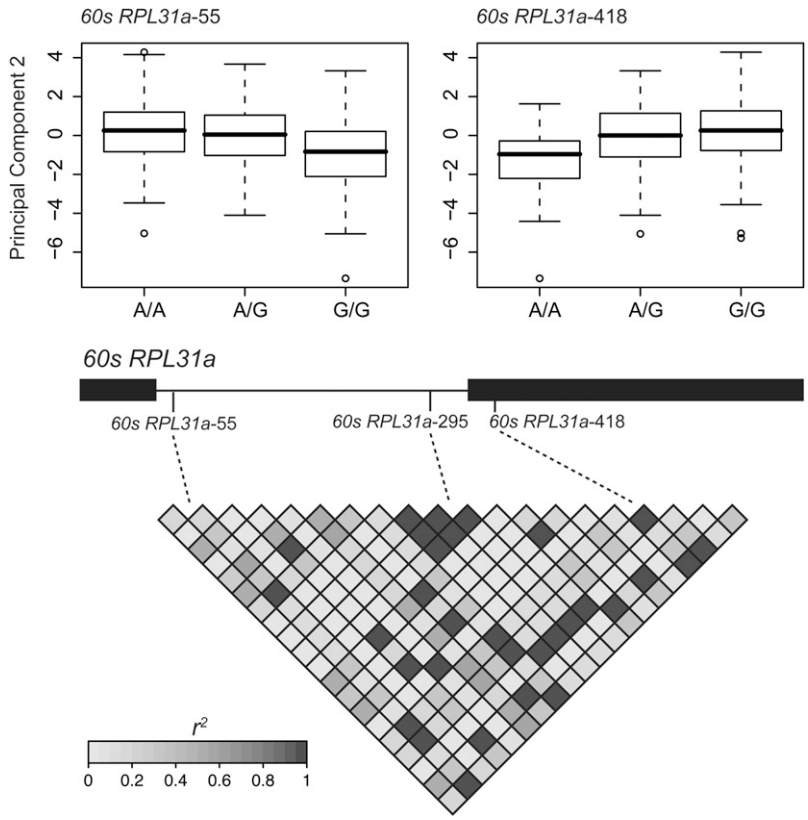


Figure 2.—An example of marker effects on the second principal component. This component is related largely to phenology and cold-tolerance traits. Each marker explains a small portion of the phenotypic variance ($r^2 \sim 3\%$) and is consistent with an additive model of gene action. Whiskers in the box plots represent 1.5 times the interquartile range. The 2 associated SNPs are in LD with one another. Illustrated are the 21 SNPs discovered for the *60s RPL31a* locus relative to the inferred gene model, as well as 3 of those 21 that were genotyped (dashed lines). Solid boxes denote exons in the gene model.

with less cold damage to stems, needles, and buds. The third principal component was related to bud burst and emergence and cold damage to needles. Two markers were associated significantly with this component, with each of those mirroring their effects on the single traits that are correlated with this component. No marker–trait associations survived multiple-test corrections for the fourth principal component.

**Modes of gene action and marker effects:** Many of the 30 SNPs associated significantly with at least one trait were consistent with modes of gene action other than codominance (Table 3). Four of the 13 marker–trait pairs (31%) for which dominance and additive effects could be calculated were consistent with over- or underdominance ($|d/a| > 1.25$). The majority of these markers were related to cold damage phenotypes, where the heterozygote had higher damage on average. The remaining 9 markers were split between modes of gene action that were additive [$|d/a| < 0.50$, $n = 4$ (31%)] or partially to fully dominant [$0.50 < |d/a| < 1.25$, $n = 5$ (38%)]. Most effects were small to moderate and accounted for only 10–85% of the phenotypic standard deviation.

The derived allele was typically the minor allele. The additive effects of these derived alleles varied by trait, but were often small to moderate in size (Table 3). The additive effects on the cold damage to stems phenotype (stmcold), for example, varied from −0.10% (*CN637339.1*-337) to 0.28% (*Pm_CL234Contig1*-156). An analysis using the minor allele for those loci without an outgroup sequence found similar effects, with the minor allele typically conveying changes in trait consistent with cold hardiness (Table 3). The relationship between the genotypic classes of a marker associated to a phenotype and the environmental gradient that affects that phenotype is consistent with this pattern (Figure 3). For example, the G allele at the *4CL1*-520 marker is the minor allele and genotypes containing this allele are found at sites with significantly lower annual average temperatures. Trees at these sites also had significantly lower cold damage to buds. The additive effect of the G allele at this marker was estimated to be −0.17% (Table 3).

**Comparison to analyses using the full data set:** Inclusion of the 57 families located east of the Cascade crest in northeastern Washington changed the association analyses dramatically. The number of significant associations increased to 668 at a nominal significance threshold of $P = 0.05$. Multiple-test corrections using the FDR method reduced this number to 164 at a significance threshold of $Q = 0.10$ (Table S5). These represent increases in the number of detected associations of 47 and 447%, respectively. These 164 associations represent 44 unique SNP markers located within 35 candidate genes. Surprisingly, the vast increase in significant results is caused by a set of 24 candidate genes, with markers in 7 of those 24 accounting for 40% of the increased number of significant tests. These 7

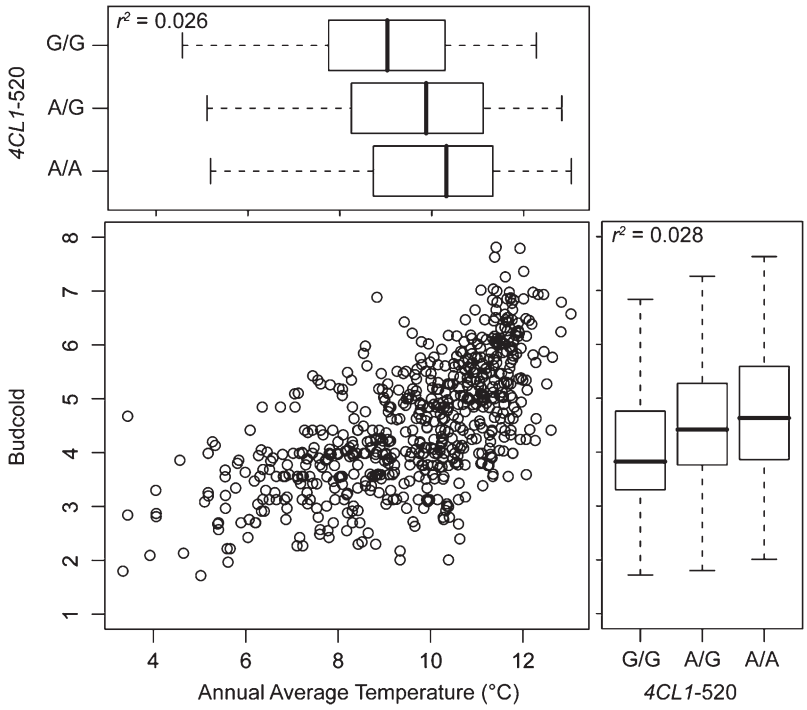


FIGURE 3.—An illustration of the relationship between phenotype, genotype, and the annual average temperature gradient (ANNAVT). Cold damage to buds is related to the annual average temperature of the locality where the source tree was sampled. This phenotype is also associated with a SNP at the *4CL1* locus. Genotypes at this SNP vary along the temperature gradient as expected, with the G allele being located at lower annual average temperatures. This SNP fits well with an additive model of gene action and is nonsynonymous.

markers also exhibit increased levels of differentiation as measured by $F_{CT}$ (Figure S3). The *Pm_CL61Contig1*-134 and *ES420757.1*-311 markers, for example, have values of $F_{CT}$ ~10-fold higher than SNPs that remained unassociated with a phenotype regardless of whether or not the 57 families were included (Figure 4). Each of these markers was associated to ~65% of the individual phenotypic traits when the 57 families in question were included in the analysis.

## DISCUSSION

**Associations and marker effects:** Temperature is the most important environmental variable with respect to adaptation by coastal Douglas fir to the Pacific Northwest environments (St. Clair *et al.* 2005; St. Clair 2006). We have identified a set of 12 candidate genes that are associated significantly with cold hardiness in coastal Douglas fir. Four of the 15 polymorphisms within these genes are nonsynonymous substitutions, with the remainder being located largely in synonymous positions. These associations represent a refined list of candidate genes for further analysis, as well as provide insight into the genetic components of complex traits in coastal Douglas fir.

All 30 significant associations accounted for a small proportion of the phenotypic variance and were within the range of those published previously for forest trees (<1%–4%; Thumma *et al.* 2005; González-Martínez *et al.* 2007, 2008; Ingvarsson *et al.* 2008), as well as other wind-pollinated species (<1%–5%; Weber *et al.* 2007, 2008). Patterns such as these reflect a polygenic quantitative model, which is supported by a long history of quantitative genetic studies in forest trees (Namkoong 1979). Several marker alleles deviate from this quantitative model, however, when effects were measured in terms of phenotypic standard deviations (Table 3). Most of the associated markers accounted for less than half of a phenotypic standard deviation. Two markers in the *60s RPL31a* candidate gene explain upward of 80% of the standard deviation for the second principal component (*60s RPL31a*-55-Prin2, $0.63\sigma_p$; *60s RPL31a*-418-Prin2, $0.83\sigma_p$). The additive effect of the latter marker on bud set, moreover, was large, with a substitution of the G allele producing a 2-day increase in the time to set bud. Such effects compounded across a few loci could explain large portions of the development of cold hardiness in coastal Douglas fir.

The cumulative effects across loci each with a small effect, however, can also be large. For example, ~20% of the phenotypic variance in early wood specific gravity was explained by a handful of SNPs in loblolly pine (González-Martínez *et al.* 2007). Here, six SNPs explain ~17% of the phenotypic variance in cold damage to stems (Table 2). Thus, analyses based on expanded sets of candidate genes have the potential to identify markers that explain a large fraction of the phenotypic variance across numerous traits.

Seventeen candidate genes were shown previously to collocate with clonally replicated spring and fall cold-hardiness QTL in coastal Douglas fir (Wheeler *et al.* 2005). Of those, 9 had SNPs genotyped within the association population. Marker–trait associations within

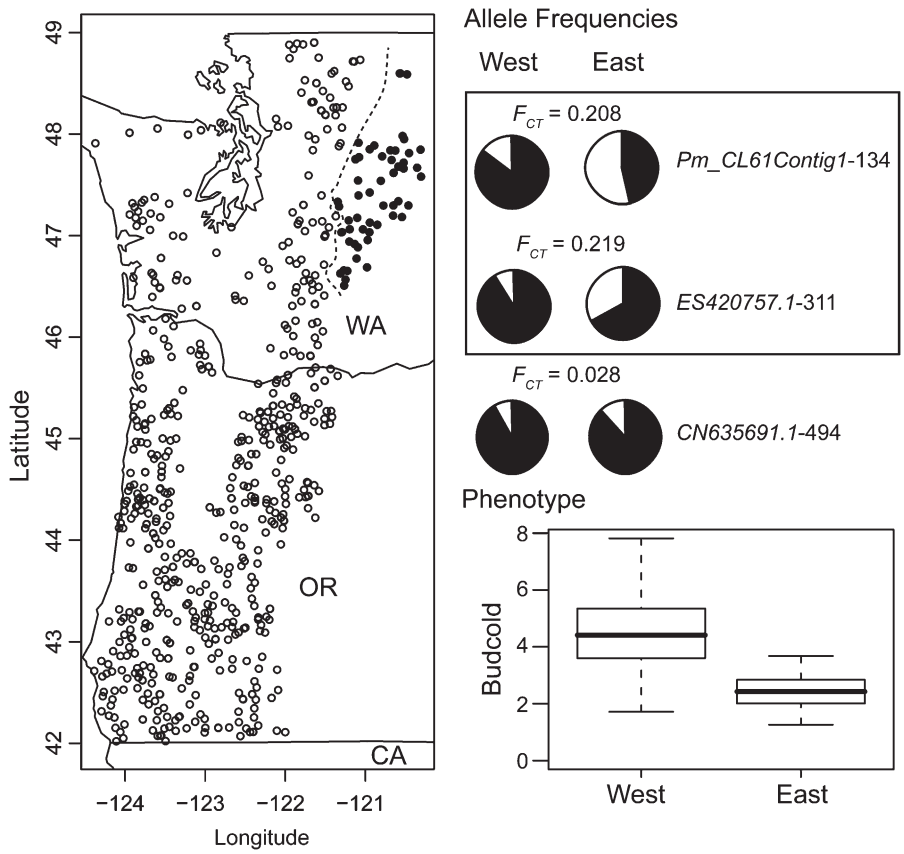


Figure 4.—The families located east of the Cascade crest in northeastern Washington affect the outcome of the association testing. Inclusion of these 57 families inflates the number of significant associations from 30 to 164. This is due mainly to a set of seven markers, each within a unique candidate gene, that show elevated levels of differentiation ($F_{CT} = 0.08$–$0.22$) across this region. Illustrated are two examples (markers enclosed in the box), with these SNPs being associated to ~65% of the traits when the 57 eastside families are included in the analysis. The global $F_{CT}$ estimate for unassociated SNP and allozyme markers is in the range of 0.019–0.035 for comparison. Pie charts denote SNP allele frequencies east (open sections) and west (solid sections) of the Cascade crest.

these 9 genes did not survive multiple-test corrections when the families east of the Cascade crest were excluded from the analysis. When these families were included, however, 2 of those 9 genes harbored markers associated with at least one cold-hardiness related trait: α-*tubulin* and *erd15*. Both candidate genes were mapped to linkage group seven and collocated with a QTL for spring needle cold hardiness (*cf.* Figure 1 in Wheeler *et al.* 2005). Markers within these candidate genes were associated with bud-set and cold-tolerance phenotypes, respectively.

More than half of the SNPs associated with cold tolerance and bud set showed modes of gene action consistent with over- or underdominance (Table 3). A trade-off exists between growth and fall cold hardiness and these two traits are often negatively correlated, both phenotypically and genetically in Douglas fir (Rehfeldt 1979; Aitken *et al.* 1996). There is also a positive genetic correlation between bud set and growth (Rehfeldt 1979, 1983; Campbell 1986; Li and Adams 1993) and a negative genetic correlation between bud set and fall cold hardiness (O'Neill *et al.* 2001). The relationships among these traits indicate that trees that set bud later have better growth (likely due to extending their growing season), but experience higher fall cold injury (lower cold hardiness), a pattern that is observed in many forest trees.

The phenotypic correlations of stem cold damage with bud set were moderate and highly significant (BS1, $F_{1,863} = 613.8$, $P < 0.001$, $r = 0.64$; BS2, $F_{1,863} = 396.5$, $P < 0.001$, $r = 0.56$; data from St. Clair *et al.* 2005 and St. Clair 2006). The correlation with height was weaker, but there was a significant correlation with height increment (HTINC: $F_{1,863} = 63.5$, $P < 0.001$, $r = 0.26$). The inferiority of heterozygotes for cold hardiness may thus be a correlated response that is a trade-off for higher growth. This explanation is consistent with the additive effects estimated for the CN637339.1-337 marker, with the G allele producing a later date of bud set (~1 day later) while at the same time being correlated with ~0.28% higher cold damage to stems (Table 3).

The large number of loci consistent with nonadditive modes of gene action is also consistent with epigenetic effects. These effects have been identified primarily in Norway spruce [*Picea abies* (L.) Karst.], where the timings of bud burst in spring, cessation of leader shoot growth in summer, and bud set in autumn are processes that are modified according to the temperature during female meiosis (Johnsen *et al.* 1996; Hänninen *et al.* 2001; Saxe *et al.* 2001; Johnsen *et al.* 2005a,b). Conditions colder than normal advance the onset of these effects, while temperatures above normal delay their onset (Kvaalen and Johnsen 2008). Further work has shown that this is not a genotypic-selection scenario and is likely to be a long-lasting epigenetic phenomenon tied to the temperature and photoperiod of the maternal tree during seed production (Besnard *et al.* 2008).

The same traits as those shown to have epigenetic effects in Norway spruce have been implicated in coastal Douglas fir as showing strong evidence for phenotypic adaptation. Given that our sampling design was sensitive primarily to additive effects (*i.e.*, we used breeding values for the phenotypes) and that many of the deviations from additive effects are strong (*cf.* Table 3), epigenetic factors warrant further investigation. This is especially true because of the importance of this species to forest ecosystems in western North America and the inferred impacts of global climate change on its abundance and distribution.

**Effects of population structure on association analyses:** Strong population differentiation along an environmental gradient provides indirect evidence of the role of natural selection in shaping adaptive phenotypic variation (Endler 1986). This has aptly been demonstrated for a variety of cold-hardiness and growth-related phenotypes in coastal Douglas fir. Similar trends for the molecular genetic variation underlying these traits have been identified here, with most SNP genotypes tracking variation along the same gradient as their associated phenotypes (Figure 3). These gradients may be confounded with a contact zone between the coastal and the interior varieties of Douglas fir. Of particular interest are the 57 families located east of the Cascade crest in northeastern Washington. Inclusion of these families increased dramatically the number of significant associations from 30 to 164.

The disparity between these numbers could be due to neutral population structure not captured by the *Q*-matrix or strong population differences reflecting adaptive differentiation between varieties or intervarietal hybrids. The two varieties are differentiated strongly at 20 isozyme loci (Li and Adams 1989). If the families located east of the Cascade crest are the interior variety or intervarietal hybrids, unaccounted for population structure could inflate the number of false positives. If this was the case, we expect many loci to be differentiated strongly between the eastside families and those located west of the Cascade crest. This is not what is observed when considering those SNPs not associated strongly when all or just the westside families are included in the analyses ($n = 184$ SNPs; global $F_{CT}$, 0.019; 95% C.I., 0.010–0.035). This value is much lower in magnitude than that estimated between the varieties using allozymes ($G_{ST} = 0.116$; Li and Adams 1989). This suggests that the *Q*-matrix utilized in the GLMs is controlling correctly for background levels of population structure. The nonsignificant global estimate of $F_{IS}$, moreover, supports our avoidance of a mixed linear model (MLM) (*cf.* Yu *et al.* 2006) approach using kinship estimates. Such an analysis was conducted for loblolly pine, with the general conclusion that the MLM and GLM approaches produced similar results due to the fact that individuals were related less than second cousins on average (González-Martínez *et al.* 2007).

It is more likely that the effects the eastside families have on the association results are those due to adaptive differentiation between populations. Seven markers cause the majority of the increase in significant association results due to the correlation between allele frequency and phenotypic differentiation across the Cascade crest. These seven markers have values of $F_{CT}$ in the range of 0.08–0.22, which is an order of magnitude greater than the background level of differentiation. Further interpretation regarding the effects of these genes relative to the phenotypes is complicated given that most of the phenotypes also differ between these families and those located west of the Cascade crest.

Palynological data support a postglacial contact between varieties emerging from distinct southern Pleistocene refugia occurring ~7000 years ago (Tsukada 1982; Wells 1983). Given that the coastal variety is more similar to the northern populations of the interior variety at allozyme loci (Li and Adams 1989), it is probable that introgression has and is occurring between varieties east of the Cascade crest. This also suggests that cold-adapted alleles identified here may have originated in the interior variety. Consistent with this hypothesis are common garden studies of intervarietal hybrids, which yield data consistent with largely nonadditive allelic effects for growth and cold-hardiness traits in the hybrids (Rehfeldt 1977). Many of the associations detected here may also reflect the correlation between freezing and drought-stress tolerance (*cf.* Blödner *et al.* 2005), especially given that water is one of the major limiting factors across the northern ranges of coastal and interior Douglas fir (Littell *et al.* 2008). Regardless of the hypothesis, it is clear that further phylogeographic investigations and association studies are needed across the entire range of coastal and interior Douglas fir.

**Functions of candidate genes:** Inferred functionality of associated candidate genes varied across trait categories. Genes associated to cold-tolerance traits had homology to loci encoding proteins involved with lignin biosynthesis and cell wall architecture, transcription regulation, and signal transduction in Arabidopsis. Similar types of genes were upregulated in Sitka spruce in response to cold temperatures (Holliday *et al.* 2008). There was a surprising lack of calcium-signaling related genes producing significant marker–trait associations. This is not the case with the inclusion of the 57 families located east of the Cascade crest. Inclusion of those families resulted in markers located within loci encoding a cysteine proteinase (*Pm_CL135Contig1*) and a cyclophilin (*Pm_CL61Contig1*) being associated with several cold-tolerance and phenology traits. Cyclophilins are involved with cysteine biosynthesis in plants and in calcium–calmodulin-activated serine/threonine-specific protein phosphatase calcineurin in humans and yeast (Wang and Heitman 2005; Dominguez-Solis *et al.* 2008).

Genes associated with phenology and emergence traits were homologous to loci encoding proteins involved with ribosome biogenesis (*60s RPL31a*) and stress tolerance (*LEA-EMBL11*). Notably, a SOUL heme-binding protein (*Pm_CL783Contig1*) had significant marker–trait associations with bud-set and root-shoot ratio. A homologous protein has been localized to vacuoles in Arabidopsis and linked putatively with hemoprotein synthesis (Jaquinod *et al.* 2007).

**Integration with population genetic results:** Patterns of polymorphism and divergence for 2 of the 12 candidate genes identified here were characterized previously as deviant from null models incorporating genetic drift and some forms of historical demography (Eckert *et al.* 2009b). The *Pm_CL234Contig1* locus has an excess of high-frequency derived polymorphisms (Fay and Wu's normalized $H = -2.35$, $P < 0.05$), a pattern that is consistent with hitchhiking, while the *ES421311.1* locus has an excess of rare alleles (Tajima's $D = -1.70$, $P < 0.05$). Both of these results, however, did not survive multiple-test corrections or incorporation of severe bottlenecks into hypothesis tests of neutrality. The association reported here for the *Pm_CL234Contig1* locus is with a marker segregating a high-frequency derived allele. This allele conveys a 1.11% reduction in cold damage to stems. A simplistic interpretation of this pattern is that the association detected here is due to the recent fixation of an advantageous haplotype containing the derived SNP allele. Future research combining population, quantitative, and landscape genetics, therefore, will offer new insights into the genetic architecture of cold-hardiness related traits in coastal Douglas fir.

**Conclusions:** We identified 30 marker–trait associations across 12 candidate genes and 10 cold-hardiness related traits. Marker effects were small ($1\% < r^2 < 3.6\%$), consistent with a polygenic quantitative model, and tracked similar environmental gradients to those affecting the phenotypes to which the markers were associated. Higher-order effects (*e.g.*, dominance) were prevalent throughout the association results, with 86% of the significant markers having nonadditive effects. Such results would benefit directly from validation in additional association populations (*cf.* Weber *et al.* 2008), especially since significant QTL-by-environment interactions have been documented for several adaptive traits (Jermstad *et al.* 2003). Nonetheless, these results represent a further step toward the dissection of cold hardiness in coastal Douglas fir.

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# Association Genetics of Coastal Douglas Fir (*Pseudotsuga menziesii* var. *menziesii*, Pinaceae). I. Cold-Hardiness Related Traits

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**TABLE S1**

**Sample localities for the 700 families used for association mapping in coastal Douglas-fir**

| Sample ID | Latitude | Longitude | Elevation (m) | ANNAVT (°C)[a] |
|---|---|---|---|---|
| 1004 | 43.238 | -123.117 | 325 | 12.15 |
| 1005 | 43.369 | -122.967 | 344 | 11.68 |
| 1006 | 43.369 | -122.967 | 344 | 11.68 |
| 1007 | 42.734 | -122.662 | 835 | 10.53 |
| 1008 | 42.718 | -122.816 | 854 | 10.63 |
| 1009 | 43.299 | -123.153 | 238 | 11.71 |
| 1011 | 43.513 | -123.178 | 488 | 11.35 |
| 1014 | 43.717 | -123.049 | 250 | 10.87 |
| 1026 | 44.414 | -122.672 | 183 | 11.12 |
| 1027 | 44.412 | -122.669 | 183 | 11.12 |
| 1029 | 44.537 | -122.517 | 777 | 9.75 |
| 1030 | 44.538 | -122.517 | 780 | 9.75 |
| 1031 | 44.525 | -122.467 | 485 | 10.30 |
| 1032 | 44.565 | -122.380 | 778 | 9.85 |
| 1043 | 44.849 | -122.363 | 659 | 8.83 |
| 1044 | 44.849 | -122.363 | 664 | 8.83 |
| 1046 | 44.849 | -122.424 | 820 | 8.72 |
| 1047 | 44.810 | -122.585 | 490 | 9.77 |
| 1048 | 44.978 | -122.546 | 610 | 9.46 |
| 1050 | 45.017 | -122.545 | 453 | 9.92 |
| 1052 | 44.987 | -122.454 | 624 | 9.14 |
| 1056 | 44.986 | -122.270 | 731 | 6.75 |
| 1066 | 45.448 | -122.150 | 247 | 10.93 |
| 1074 | 46.506 | -122.172 | 232 | 10.01 |
| 1079 | 46.358 | -122.518 | 479 | 9.69 |
| 1080 | 46.358 | -122.518 | 477 | 9.69 |
| 1081 | 46.408 | -122.626 | 184 | 9.92 |
| 1084 | 46.672 | -122.208 | 652 | 8.43 |
| 1087 | 47.147 | -121.823 | 425 | 7.76 |
| 1094 | 47.130 | -121.658 | 838 | 7.06 |
| 1095 | 47.593 | -121.709 | 446 | 8.59 |
| 1098 | 47.949 | -121.625 | 491 | 6.87 |
| 1100 | 42.255 | -122.390 | 1427 | 6.22 |
| 1103 | 45.300 | -121.752 | 1177 | 5.28 |
| 1105 | 46.764 | -121.799 | 945 | 4.05 |
| 1106 | 48.314 | -121.672 | 871 | 9.01 |

| | | | | |
|---|---|---|---|---|
| 1111 | 48.884 | -121.777 | 737 | 5.34 |
| 1112 | 47.910 | -121.311 | 532 | 5.66 |
| 1113 | 47.881 | -121.330 | 718 | 5.99 |
| 1114 | 48.715 | -121.139 | 305 | 7.90 |
| 1117 | 43.200 | -124.200 | 68 | 11.43 |
| 1118 | 43.221 | -123.907 | 413 | 11.77 |
| 1119 | 43.200 | -124.050 | 209 | 12.14 |
| 1120 | 42.998 | -123.737 | 410 | 11.56 |
| 1126 | 45.823 | -122.017 | 549 | 8.34 |
| 1127 | 45.891 | -122.185 | 748 | 8.93 |
| 1128 | 46.608 | -122.455 | 466 | 8.92 |
| 1132 | 47.986 | -121.708 | 382 | 8.33 |
| 1133 | 47.842 | -121.655 | 108 | 9.26 |
| 1134 | 48.531 | -121.755 | 63 | 9.88 |
| 1136 | 48.732 | -121.064 | 541 | 8.32 |
| 1139 | 48.716 | -121.155 | 311 | 8.02 |
| 1141 | 48.318 | -121.649 | 559 | 9.14 |
| 1144 | 47.229 | -121.908 | 447 | 9.18 |
| 1146 | 47.113 | -121.843 | 1023 | 6.89 |
| 1147 | 47.117 | -121.842 | 977 | 6.89 |
| 1149 | 47.303 | -121.774 | 426 | 8.70 |
| 1150 | 47.302 | -121.772 | 424 | 8.70 |
| 1154 | 47.287 | -121.328 | 939 | 4.65 |
| 1159 | 47.334 | -121.344 | 808 | 4.77 |
| 1163 | 47.423 | -121.411 | 924 | 4.33 |
| 1171 | 42.835 | -122.918 | 815 | 10.07 |
| 1172 | 42.891 | -122.832 | 1040 | 9.41 |
| 1175 | 47.751 | -121.123 | 1060 | 4.06 |
| 1176 | 47.753 | -121.124 | 1169 | 4.06 |
| 1177 | 47.773 | -121.078 | 1016 | 3.60 |
| 1191 | 44.623 | -123.546 | 244 | 10.72 |
| 1195 | 44.332 | -123.860 | 292 | 12.19 |
| 1197 | 44.342 | -123.837 | 189 | 12.27 |
| 1199 | 44.272 | -122.861 | 487 | 11.27 |
| 1200 | 44.620 | -122.660 | 570 | 9.95 |
| 1201 | 44.620 | -122.660 | 570 | 9.95 |
| 1202 | 44.602 | -121.948 | 792 | 7.98 |
| 1207 | 44.969 | -122.587 | 548 | 9.73 |
| 1208 | 45.116 | -122.204 | 1089 | 7.67 |
| 1210 | 45.077 | -122.076 | 539 | 9.91 |
| 1213 | 45.265 | -122.217 | 426 | 10.64 |

| | | | | |
|---|---|---|---|---|
| 1214 | 45.265 | -122.214 | 427 | 10.64 |
| 1215 | 43.771 | -122.346 | 730 | 10.59 |
| 1219 | 43.325 | -122.192 | 1301 | 7.22 |
| 1220 | 43.324 | -122.190 | 1310 | 7.22 |
| 1222 | 43.239 | -122.336 | 1137 | 7.88 |
| 1223 | 44.191 | -122.015 | 874 | 9.15 |
| 1224 | 44.194 | -122.017 | 828 | 9.15 |
| 1229 | 45.755 | -123.815 | 122 | 10.27 |
| 1230 | 45.755 | -123.815 | 122 | 10.27 |
| 1232 | 45.771 | -123.723 | 391 | 9.85 |
| 1233 | 45.624 | -123.824 | 75 | 10.53 |
| 1236 | 46.182 | -123.494 | 119 | 10.18 |
| 1237 | 46.108 | -123.394 | 353 | 9.77 |
| 1238 | 46.067 | -123.649 | 305 | 9.43 |
| 1239 | 45.983 | -123.513 | 285 | 9.55 |
| 1240 | 45.834 | -123.750 | 244 | 10.01 |
| 1241 | 45.836 | -123.750 | 232 | 10.01 |
| 1242 | 45.780 | -123.685 | 286 | 9.90 |
| 1243 | 45.908 | -123.460 | 244 | 9.52 |
| 1245 | 45.806 | -123.420 | 609 | 8.96 |
| 1246 | 45.935 | -123.063 | 366 | 9.10 |
| 1247 | 46.307 | -123.110 | 347 | 8.97 |
| 1248 | 46.306 | -123.108 | 347 | 8.97 |
| 1249 | 46.281 | -123.292 | 243 | 9.91 |
| 1250 | 46.397 | -123.659 | 229 | 9.83 |
| 1251 | 46.586 | -123.896 | 30 | 10.39 |
| 1252 | 46.540 | -123.629 | 120 | 9.62 |
| 1253 | 46.561 | -123.363 | 244 | 9.60 |
| 1254 | 46.611 | -123.345 | 290 | 9.71 |
| 1255 | 47.314 | -123.450 | 246 | 8.88 |
| 1258 | 47.057 | -123.688 | 61 | 10.16 |
| 1259 | 47.147 | -123.779 | 61 | 10.14 |
| 1260 | 47.218 | -123.680 | 183 | 9.41 |
| 1261 | 47.352 | -123.762 | 303 | 9.01 |
| 1262 | 47.380 | -123.630 | 243 | 8.70 |
| 1263 | 47.181 | -123.560 | 61 | 9.84 |
| 1264 | 46.992 | -123.275 | 143 | 10.68 |
| 2009 | 45.394 | -121.860 | 669 | 7.44 |
| 2010 | 45.004 | -122.030 | 762 | 9.10 |
| 2011 | 45.091 | -122.004 | 714 | 8.31 |
| 2012 | 45.018 | -121.926 | 673 | 7.21 |

| | | | | |
|---|---|---|---|---|
| 2017 | 45.121 | -121.910 | 960 | 6.36 |
| 2019 | 45.099 | -122.026 | 961 | 8.89 |
| 2022 | 44.893 | -121.903 | 1088 | 6.51 |
| 2023 | 45.415 | -121.888 | 983 | 7.45 |
| 2024 | 44.909 | -122.012 | 795 | 7.07 |
| 2029 | 43.287 | -122.614 | 725 | 10.53 |
| 2034 | 43.217 | -122.199 | 1604 | 6.34 |
| 2036 | 43.246 | -122.236 | 1274 | 7.16 |
| 2037 | 43.186 | -122.725 | 1427 | 9.74 |
| 2038 | 43.195 | -122.651 | 1366 | 7.93 |
| 2039 | 43.165 | -122.301 | 1719 | 5.19 |
| 2046 | 43.142 | -122.795 | 1029 | 11.30 |
| 2051 | 43.131 | -122.881 | 1276 | 10.15 |
| 2052 | 43.146 | -122.664 | 1197 | 8.90 |
| 2056 | 43.139 | -122.497 | 1136 | 7.62 |
| 2058 | 43.131 | -122.515 | 1133 | 7.62 |
| 2060 | 43.567 | -122.728 | 816 | 10.22 |
| 2069 | 43.424 | -122.417 | 1120 | 8.02 |
| 2089 | 43.582 | -122.381 | 1417 | 8.31 |
| 2093 | 44.270 | -122.124 | 1185 | 7.58 |
| 3002 | 45.232 | -122.527 | 160 | 11.31 |
| 3003 | 45.324 | -122.150 | 511 | 10.39 |
| 3004 | 45.323 | -122.154 | 551 | 10.39 |
| 3007 | 45.209 | -122.450 | 288 | 11.04 |
| 3009 | 45.191 | -122.342 | 386 | 10.37 |
| 3010 | 45.043 | -122.249 | 1105 | 5.85 |
| 3011 | 45.120 | -122.327 | 927 | 8.00 |
| 3012 | 45.122 | -122.326 | 942 | 8.00 |
| 3013 | 45.066 | -122.283 | 955 | 6.37 |
| 3023 | 44.734 | -122.465 | 373 | 10.24 |
| 3024 | 44.736 | -122.459 | 364 | 10.24 |
| 3027 | 44.513 | -122.687 | 318 | 10.62 |
| 3029 | 44.740 | -122.611 | 611 | 10.29 |
| 3031 | 44.783 | -122.558 | 440 | 10.09 |
| 3036 | 44.123 | -122.516 | 426 | 11.29 |
| 3042 | 43.799 | -122.884 | 725 | 10.70 |
| 3050 | 44.323 | -122.942 | 431 | 11.33 |
| 3051 | 44.325 | -122.943 | 413 | 11.33 |
| 3052 | 44.300 | -122.638 | 451 | 10.56 |
| 3053 | 44.150 | -122.635 | 610 | 10.99 |
| 3056 | 44.278 | -122.810 | 372 | 11.43 |

| | | | | |
|---|---|---|---|---|
| 3059 | 44.029 | -122.763 | 608 | 11.24 |
| 3066 | 43.564 | -122.991 | 751 | 11.13 |
| 3069 | 43.353 | -122.907 | 1223 | 11.51 |
| 3070 | 43.715 | -122.832 | 807 | 10.75 |
| 3071 | 43.455 | -122.891 | 492 | 11.36 |
| 3073 | 43.387 | -122.753 | 673 | 10.87 |
| 3075 | 43.282 | -123.004 | 793 | 11.53 |
| 3076 | 43.223 | -123.006 | 569 | 11.81 |
| 3079 | 43.209 | -122.940 | 728 | 11.74 |
| 3081 | 43.287 | -122.964 | 576 | 11.34 |
| 3082 | 43.380 | -123.213 | 289 | 11.74 |
| 3083 | 43.379 | -123.212 | 286 | 11.74 |
| 3084 | 43.363 | -123.234 | 389 | 11.78 |
| 3086 | 43.203 | -123.132 | 557 | 12.61 |
| 3088 | 43.492 | -123.066 | 365 | 12.07 |
| 3090 | 42.916 | -123.187 | 586 | 11.74 |
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| 3099 | 43.114 | -123.066 | 744 | 12.29 |
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| 3106 | 42.980 | -122.954 | 865 | 11.35 |
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| 3112 | 42.433 | -122.526 | 1209 | 9.07 |
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| 3125 | 42.146 | -122.487 | 1402 | 7.03 |
| 3128 | 42.071 | -122.560 | 1189 | 8.34 |
| 3129 | 42.112 | -122.399 | 1159 | 8.09 |
| 3130 | 42.112 | -122.402 | 1156 | 8.09 |
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| 3136 | 42.208 | -122.579 | 1204 | 8.80 |
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| 3140 | 42.124 | -122.105 | 1463 | 7.60 |
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| 3145 | 45.234 | -123.513 | 729 | 10.07 |
| 3147 | 44.320 | -123.524 | 663 | 11.34 |
| 3148 | 44.321 | -123.522 | 655 | 11.34 |
| 3153 | 43.154 | -123.695 | 753 | 10.99 |
| 3154 | 42.241 | -123.006 | 714 | 10.92 |
| 3155 | 42.264 | -123.318 | 621 | 10.21 |
| 3156 | 42.259 | -123.328 | 675 | 10.21 |

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| 3157 | 42.154 | -122.837 | 1205 | 7.82 |
| 3158 | 42.151 | -122.836 | 1148 | 7.82 |
| 3161 | 45.710 | -122.958 | 426 | 9.59 |
| 3162 | 45.710 | -122.954 | 415 | 9.59 |
| 3163 | 45.354 | -123.384 | 540 | 9.46 |
| 3164 | 45.352 | -123.385 | 613 | 9.46 |
| 3167 | 42.989 | -123.439 | 679 | 11.55 |
| 3169 | 43.291 | -123.944 | 317 | 11.85 |
| 3170 | 43.120 | -123.933 | 193 | 11.90 |
| 3171 | 43.786 | -123.135 | 459 | 11.13 |
| 3172 | 43.786 | -123.129 | 452 | 11.13 |
| 3173 | 43.871 | -123.291 | 326 | 11.52 |
| 3176 | 43.772 | -123.230 | 304 | 11.50 |
| 3178 | 43.737 | -123.401 | 417 | 11.11 |
| 3179 | 43.738 | -123.403 | 410 | 11.11 |
| 3180 | 43.033 | -123.919 | 405 | 11.74 |
| 3181 | 43.457 | -123.622 | 351 | 11.57 |
| 3182 | 43.459 | -123.622 | 378 | 11.57 |
| 3183 | 43.540 | -123.473 | 250 | 11.71 |
| 3184 | 43.541 | -123.473 | 263 | 11.71 |
| 3186 | 43.589 | -123.332 | 231 | 11.62 |
| 3187 | 43.337 | -123.553 | 669 | 12.06 |
| 3188 | 43.288 | -123.600 | 677 | 12.01 |
| 3189 | 45.685 | -123.039 | 328 | 10.07 |
| 3190 | 45.684 | -123.042 | 294 | 10.07 |
| 3191 | 42.986 | -123.441 | 731 | 11.55 |
| 3195 | 43.918 | -123.707 | 536 | 11.08 |
| 3196 | 43.920 | -123.710 | 577 | 11.08 |
| 3197 | 44.067 | -123.647 | 624 | 11.48 |
| 3198 | 44.068 | -123.649 | 635 | 11.48 |
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| 3200 | 45.281 | -123.472 | 853 | 9.39 |
| 3205 | 43.069 | -124.008 | 371 | 11.94 |
| 3206 | 43.297 | -123.831 | 480 | 11.80 |
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| 3209 | 43.038 | -123.911 | 409 | 11.74 |
| 3212 | 43.561 | -123.775 | 303 | 11.77 |
| 3213 | 43.560 | -123.775 | 298 | 11.77 |
| 3215 | 43.691 | -123.603 | 243 | 11.59 |
| 3216 | 43.691 | -123.603 | 243 | 11.59 |
| 3218 | 43.319 | -124.073 | 87 | 11.66 |

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| 3222 | 43.297 | -123.815 | 442 | 11.77 |
| 3223 | 43.134 | -123.754 | 677 | 10.90 |
| 3224 | 42.998 | -124.069 | 237 | 11.92 |
| 3225 | 42.907 | -124.148 | 194 | 11.67 |
| 3228 | 43.836 | -123.502 | 306 | 11.30 |
| 3233 | 44.213 | -123.608 | 548 | 11.61 |
| 3234 | 44.118 | -123.748 | 122 | 11.90 |
| 3235 | 44.023 | -123.621 | 288 | 11.43 |
| 3238 | 43.828 | -123.354 | 217 | 11.38 |
| 3239 | 43.871 | -123.184 | 323 | 11.21 |
| 3240 | 44.239 | -123.438 | 485 | 12.07 |
| 3241 | 44.145 | -123.479 | 252 | 11.63 |
| 3242 | 43.966 | -123.428 | 335 | 11.36 |
| 3243 | 43.965 | -123.573 | 258 | 11.44 |
| 3246 | 44.099 | -123.521 | 314 | 11.43 |
| 3248 | 42.690 | -123.816 | 418 | 11.61 |
| 3249 | 42.692 | -123.817 | 394 | 11.61 |
| 3250 | 42.767 | -123.771 | 855 | 10.89 |
| 3251 | 42.740 | -123.848 | 399 | 11.71 |
| 3253 | 42.678 | -123.646 | 714 | 10.70 |
| 3255 | 42.620 | -123.782 | 730 | 10.65 |
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| 3259 | 42.592 | -123.666 | 1014 | 11.08 |
| 3261 | 42.649 | -123.405 | 423 | 9.48 |
| 3262 | 42.484 | -123.567 | 655 | 11.09 |
| 3263 | 42.482 | -123.563 | 608 | 11.09 |
| 3264 | 42.127 | -123.653 | 426 | 11.79 |
| 3265 | 42.261 | -123.474 | 499 | 10.79 |
| 3268 | 42.226 | -123.372 | 772 | 9.47 |
| 3269 | 42.348 | -123.515 | 384 | 11.26 |
| 3270 | 42.317 | -123.589 | 491 | 11.31 |
| 3271 | 42.073 | -123.593 | 634 | 11.44 |
| 3272 | 42.145 | -123.509 | 631 | 11.04 |
| 3273 | 42.227 | -123.604 | 504 | 11.53 |
| 3275 | 42.657 | -123.446 | 441 | 9.61 |
| 3281 | 42.618 | -123.199 | 852 | 11.16 |
| 3286 | 42.475 | -123.118 | 493 | 11.94 |
| 3288 | 42.541 | -123.292 | 614 | 10.88 |
| 3291 | 42.383 | -123.192 | 689 | 11.19 |
| 3294 | 42.664 | -123.189 | 850 | 10.90 |

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| 3295 | 42.753 | -123.133 | 1206 | 10.36 |
| 3298 | 42.753 | -123.144 | 1219 | 10.36 |
| 3300 | 42.212 | -123.125 | 664 | 10.38 |
| 3301 | 42.152 | -122.997 | 625 | 9.62 |
| 3302 | 42.285 | -123.058 | 746 | 10.71 |
| 3303 | 42.148 | -123.231 | 750 | 9.70 |
| 3310 | 42.107 | -123.006 | 748 | 8.29 |
| 3312 | 43.614 | -123.497 | 463 | 11.79 |
| 3313 | 43.708 | -123.504 | 275 | 11.49 |
| 3314 | 43.393 | -123.588 | 301 | 11.65 |
| 3316 | 43.585 | -123.411 | 483 | 11.21 |
| 3318 | 43.609 | -123.381 | 246 | 11.27 |
| 3324 | 42.841 | -123.561 | 584 | 10.81 |
| 3326 | 42.836 | -123.463 | 584 | 10.95 |
| 3327 | 42.837 | -123.460 | 660 | 10.95 |
| 3328 | 42.895 | -123.609 | 563 | 10.82 |
| 3329 | 43.015 | -123.477 | 414 | 11.49 |
| 3330 | 43.017 | -123.479 | 369 | 11.49 |
| 3332 | 45.652 | -122.913 | 344 | 10.52 |
| 3333 | 45.872 | -123.056 | 427 | 8.82 |
| 3334 | 45.829 | -123.078 | 345 | 8.88 |
| 3335 | 45.725 | -123.062 | 309 | 9.41 |
| 3340 | 45.244 | -123.427 | 543 | 9.74 |
| 3341 | 45.301 | -123.449 | 732 | 9.04 |
| 3344 | 45.495 | -123.778 | 475 | 10.42 |
| 3346 | 45.478 | -123.279 | 289 | 10.18 |
| 3347 | 44.876 | -123.458 | 268 | 9.92 |
| 3348 | 44.835 | -123.599 | 427 | 8.93 |
| 3349 | 44.982 | -123.433 | 245 | 10.27 |
| 3350 | 44.803 | -123.637 | 362 | 9.66 |
| 3352 | 44.954 | -123.723 | 764 | 7.56 |
| 3353 | 44.966 | -123.801 | 754 | 8.62 |
| 3354 | 44.733 | -123.538 | 201 | 9.80 |
| 3355 | 44.825 | -123.493 | 579 | 9.31 |
| 3356 | 45.002 | -123.530 | 322 | 10.02 |
| 3358 | 44.367 | -123.457 | 577 | 11.86 |
| 3360 | 44.412 | -123.695 | 563 | 10.73 |
| 3361 | 44.459 | -123.470 | 244 | 10.95 |
| 3362 | 43.610 | -123.378 | 245 | 11.27 |
| 3364 | 44.182 | -123.613 | 548 | 11.61 |
| 3365 | 43.966 | -123.573 | 298 | 11.44 |

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| 4002 | 43.761 | -121.799 | 1386 | 5.96 |
| 4004 | 44.436 | -121.710 | 974 | 7.61 |
| 4005 | 44.435 | -121.715 | 997 | 7.61 |
| 4006 | 44.564 | -121.715 | 1219 | 7.11 |
| 4010 | 44.573 | -121.727 | 1341 | 6.71 |
| 4011 | 44.485 | -121.580 | 1327 | 6.84 |
| 4012 | 44.497 | -121.766 | 1340 | 6.21 |
| 4013 | 44.222 | -121.625 | 1249 | 6.90 |
| 4015 | 44.316 | -121.715 | 1278 | 6.40 |
| 4016 | 45.073 | -122.070 | 490 | 9.94 |
| 4017 | 45.507 | -121.633 | 639 | 7.92 |
| 4018 | 44.994 | -122.065 | 524 | 8.97 |
| 4021 | 45.309 | -121.941 | 551 | 9.03 |
| 4023 | 45.505 | -121.635 | 675 | 7.92 |
| 4024 | 45.200 | -122.152 | 721 | 9.81 |
| 4027 | 45.549 | -122.114 | 729 | 9.84 |
| 4028 | 45.167 | -122.260 | 848 | 8.88 |
| 4033 | 45.124 | -122.140 | 934 | 8.87 |
| 4034 | 45.005 | -122.032 | 712 | 9.10 |
| 4036 | 44.942 | -122.188 | 1022 | 7.45 |
| 4038 | 45.237 | -122.060 | 1147 | 7.39 |
| 4039 | 45.227 | -121.767 | 1058 | 5.74 |
| 4040 | 45.336 | -121.853 | 1142 | 7.22 |
| 4041 | 45.094 | -121.878 | 1185 | 6.66 |
| 4042 | 45.092 | -121.878 | 1098 | 6.66 |
| 4046 | 45.645 | -121.679 | 975 | 8.18 |
| 4047 | 45.552 | -121.992 | 975 | 8.40 |
| 4050 | 45.048 | -121.849 | 996 | 6.10 |
| 4051 | 45.123 | -121.956 | 1177 | 6.28 |
| 4052 | 44.933 | -121.984 | 1113 | 6.59 |
| 4055 | 45.348 | -121.586 | 1132 | 5.78 |
| 4056 | 45.024 | -121.754 | 1157 | 5.11 |
| 4060 | 45.336 | -122.040 | 981 | 8.81 |
| 4066 | 45.212 | -121.966 | 1279 | 6.81 |
| 4067 | 45.146 | -121.442 | 801 | 9.04 |
| 4068 | 45.570 | -121.506 | 755 | 8.42 |
| 4069 | 45.572 | -121.508 | 742 | 8.42 |
| 4071 | 45.214 | -121.416 | 792 | 9.07 |
| 4073 | 45.167 | -121.639 | 1036 | 7.12 |
| 4074 | 45.134 | -121.511 | 1032 | 8.60 |
| 4076 | 45.196 | -121.510 | 1037 | 8.13 |

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| 4077 | 45.133 | -121.509 | 1029 | 8.60 |
| 4083 | 45.273 | -121.428 | 971 | 8.47 |
| 4084 | 45.271 | -121.483 | 1277 | 7.34 |
| 4085 | 45.283 | -121.680 | 1249 | 5.41 |
| 4088 | 42.811 | -122.481 | 887 | 8.59 |
| 4095 | 42.735 | -122.330 | 1248 | 6.31 |
| 4096 | 43.091 | -122.252 | 1560 | 5.41 |
| 4097 | 42.852 | -122.288 | 1648 | 4.57 |
| 4101 | 43.106 | -122.316 | 1518 | 5.90 |
| 4103 | 42.484 | -122.413 | 996 | 8.74 |
| 4104 | 42.482 | -122.412 | 1007 | 8.74 |
| 4106 | 42.611 | -122.384 | 1257 | 7.14 |
| 4107 | 42.551 | -122.357 | 1228 | 7.30 |
| 4108 | 42.345 | -122.386 | 1470 | 7.12 |
| 4109 | 42.430 | -122.368 | 1481 | 6.86 |
| 4110 | 42.155 | -122.701 | 1050 | 8.83 |
| 4112 | 42.827 | -122.658 | 896 | 8.63 |
| 4116 | 43.733 | -122.695 | 483 | 10.27 |
| 4118 | 43.669 | -122.716 | 537 | 9.98 |
| 4119 | 43.152 | -122.965 | 624 | 11.58 |
| 4130 | 43.331 | -122.663 | 1122 | 10.48 |
| 4131 | 43.353 | -122.386 | 1163 | 8.09 |
| 4133 | 43.219 | -122.337 | 1461 | 7.25 |
| 4136 | 43.020 | -122.713 | 740 | 11.14 |
| 4137 | 43.024 | -122.871 | 849 | 11.11 |
| 4138 | 43.027 | -122.871 | 877 | 11.11 |
| 4139 | 43.071 | -122.622 | 914 | 10.30 |
| 4140 | 43.091 | -122.583 | 897 | 9.55 |
| 4146 | 43.638 | -122.420 | 565 | 10.60 |
| 4150 | 43.948 | -122.552 | 554 | 10.92 |
| 4153 | 43.527 | -122.430 | 913 | 9.69 |
| 4156 | 43.827 | -122.529 | 915 | 10.12 |
| 4157 | 43.795 | -122.507 | 731 | 10.60 |
| 4158 | 43.746 | -122.547 | 630 | 10.20 |
| 4159 | 44.009 | -122.612 | 631 | 10.96 |
| 4160 | 43.901 | -122.338 | 734 | 10.31 |
| 4168 | 44.159 | -122.137 | 487 | 9.14 |
| 4172 | 43.959 | -122.055 | 853 | 8.16 |
| 4173 | 44.207 | -122.286 | 846 | 9.79 |
| 4174 | 44.076 | -122.244 | 732 | 9.61 |
| 4176 | 44.119 | -122.033 | 668 | 8.43 |

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| 4177 | 44.256 | -122.025 | 878 | 9.04 |
| 4178 | 44.223 | -122.052 | 677 | 9.19 |
| 4179 | 44.358 | -121.992 | 934 | 8.20 |
| 4180 | 44.186 | -122.166 | 614 | 9.22 |
| 4181 | 44.185 | -122.169 | 611 | 9.22 |
| 4182 | 44.299 | -122.192 | 1016 | 8.07 |
| 4192 | 44.366 | -122.237 | 848 | 8.05 |
| 4193 | 44.402 | -122.232 | 642 | 8.55 |
| 4194 | 44.373 | -122.380 | 648 | 9.68 |
| 4196 | 44.433 | -122.425 | 677 | 10.30 |
| 4199 | 44.418 | -122.379 | 713 | 9.92 |
| 4200 | 44.368 | -122.500 | 788 | 10.00 |
| 4201 | 44.388 | -122.379 | 609 | 9.77 |
| 4202 | 44.667 | -122.114 | 843 | 9.33 |
| 4203 | 44.791 | -122.052 | 801 | 8.42 |
| 4204 | 44.383 | -122.139 | 1332 | 7.27 |
| 4205 | 44.432 | -122.002 | 1001 | 7.90 |
| 4209 | 44.558 | -122.042 | 1158 | 8.11 |
| 4211 | 44.505 | -122.000 | 1028 | 7.65 |
| 4214 | 42.725 | -122.101 | 1372 | 6.26 |
| 4218 | 42.525 | -122.089 | 1334 | 6.19 |
| 4221 | 42.447 | -122.170 | 1344 | 6.51 |
| 5001 | 46.609 | -121.627 | 866 | 8.31 |
| 5003 | 46.492 | -121.859 | 882 | 8.99 |
| 5009 | 45.860 | -121.901 | 672 | 8.69 |
| 5013 | 45.815 | -121.809 | 771 | 8.56 |
| 5014 | 46.011 | -121.901 | 889 | 7.73 |
| 5019 | 45.818 | -121.687 | 605 | 8.31 |
| 5020 | 45.813 | -121.685 | 609 | 8.31 |
| 5025 | 45.995 | -121.629 | 1190 | 7.23 |
| 5028 | 45.922 | -121.595 | 1185 | 7.24 |
| 5029 | 48.192 | -121.500 | 272 | 8.25 |
| 5030 | 48.189 | -121.497 | 248 | 8.25 |
| 5031 | 48.901 | -121.640 | 792 | 5.56 |
| 5032 | 48.903 | -121.638 | 798 | 5.56 |
| 5033 | 47.677 | -120.556 | 1042 | 7.97 |
| 5034 | 47.677 | -120.560 | 1103 | 7.97 |
| 5035 | 47.745 | -120.672 | 601 | 7.69 |
| 5036 | 46.653 | -121.225 | 1247 | 4.93 |
| 5037 | 46.654 | -121.230 | 1268 | 4.93 |
| 5040 | 46.714 | -121.517 | 925 | 6.37 |

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| 5041 | 46.743 | -122.280 | 846 | 8.84 |
| 5043 | 46.462 | -121.661 | 1216 | 6.34 |
| 5044 | 48.263 | -121.361 | 296 | 7.75 |
| 5045 | 48.262 | -121.356 | 297 | 7.75 |
| 5050 | 48.502 | -121.610 | 517 | 9.34 |
| 5051 | 48.503 | -121.613 | 531 | 9.34 |
| 5054 | 48.753 | -121.583 | 621 | 7.80 |
| 5055 | 48.892 | -121.853 | 757 | 5.62 |
| 5057 | 48.597 | -121.421 | 689 | 9.43 |
| 5058 | 48.232 | -121.547 | 734 | 9.08 |
| 5059 | 48.233 | -121.553 | 730 | 9.08 |
| 5060 | 46.442 | -121.873 | 397 | 8.55 |
| 5061 | 46.553 | -121.767 | 311 | 8.09 |
| 5063 | 46.758 | -121.947 | 746 | 6.47 |
| 5064 | 46.496 | -121.583 | 1006 | 6.43 |
| 5065 | 46.410 | -121.513 | 1123 | 7.19 |
| 5066 | 46.527 | -121.663 | 1040 | 7.32 |
| 5068 | 46.723 | -121.858 | 767 | 6.07 |
| 5069 | 46.631 | -121.743 | 923 | 7.45 |
| 5070 | 46.355 | -121.798 | 808 | 7.48 |
| 5073 | 46.327 | -121.631 | 852 | 8.18 |
| 5074 | 46.324 | -121.629 | 853 | 8.18 |
| 5075 | 46.308 | -121.912 | 1084 | 6.94 |
| 5078 | 46.085 | -121.975 | 435 | 9.48 |
| 5079 | 46.119 | -122.004 | 382 | 9.66 |
| 5080 | 46.162 | -121.871 | 513 | 8.65 |
| 5090 | 46.058 | -121.530 | 826 | 7.78 |
| 5091 | 46.132 | -121.663 | 1269 | 7.92 |
| 5092 | 46.148 | -121.601 | 1259 | 7.17 |
| 5095 | 48.621 | -121.388 | 686 | 9.22 |
| 5096 | 48.425 | -121.530 | 568 | 9.04 |
| 5097 | 48.471 | -121.209 | 484 | 8.00 |
| 5098 | 48.748 | -121.946 | 706 | 6.05 |
| 5100 | 48.261 | -121.398 | 415 | 7.83 |
| 5104 | 48.519 | -121.242 | 723 | 8.20 |
| 5105 | 48.409 | -121.798 | 636 | 8.43 |
| 5107 | 48.839 | -121.897 | 683 | 5.19 |
| 5108 | 48.064 | -121.292 | 630 | 5.60 |
| 5111 | 48.048 | -121.476 | 697 | 6.63 |
| 5112 | 48.186 | -121.372 | 656 | 6.80 |
| 5113 | 48.359 | -121.440 | 862 | 8.52 |

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| 5114 | 48.263 | -121.284 | 669 | 7.85 |
| 5115 | 47.396 | -121.548 | 495 | 5.55 |
| 5116 | 47.396 | -121.547 | 496 | 5.55 |
| 5119 | 47.045 | -121.754 | 1286 | 5.39 |
| 5123 | 46.991 | -121.510 | 995 | 3.44 |
| 5124 | 46.991 | -121.508 | 1138 | 3.44 |
| 5125 | 46.937 | -121.958 | 992 | 6.54 |
| 5126 | 47.008 | -121.499 | 1351 | 3.34 |
| 5128 | 47.082 | -121.455 | 1232 | 3.93 |
| 5131 | 47.141 | -121.539 | 928 | 5.82 |
| 5134 | 47.191 | -121.369 | 1011 | 5.03 |
| 5135 | 47.581 | -120.305 | 1099 | 8.68 |
| 5136 | 47.669 | -120.354 | 962 | 8.01 |
| 5137 | 47.813 | -120.454 | 1195 | 7.34 |
| 5138 | 47.847 | -120.311 | 1449 | 7.51 |
| 5140 | 47.297 | -120.462 | 1687 | 4.65 |
| 5141 | 47.719 | -120.522 | 1356 | 7.17 |
| 5142 | 47.339 | -120.588 | 1296 | 4.40 |
| 5144 | 47.781 | -120.790 | 872 | 6.89 |
| 5145 | 47.915 | -121.085 | 967 | 5.25 |
| 5146 | 47.851 | -120.945 | 662 | 6.34 |
| 5147 | 47.888 | -120.888 | 609 | 6.77 |
| 5148 | 47.840 | -120.688 | 609 | 7.53 |
| 5149 | 47.838 | -120.625 | 789 | 6.71 |
| 5150 | 47.540 | -120.807 | 980 | 4.57 |
| 5152 | 47.951 | -120.516 | 1069 | 7.04 |
| 5153 | 47.982 | -120.535 | 1222 | 6.94 |
| 5154 | 47.197 | -120.654 | 1004 | 5.60 |
| 5156 | 47.396 | -121.086 | 818 | 4.33 |
| 5157 | 47.107 | -120.851 | 797 | 5.81 |
| 5159 | 47.128 | -120.944 | 1163 | 5.36 |
| 5160 | 47.297 | -120.650 | 1107 | 4.50 |
| 5161 | 47.034 | -120.949 | 1310 | 3.57 |
| 5163 | 47.296 | -120.775 | 1344 | 4.53 |
| 5164 | 47.062 | -121.030 | 1656 | 3.35 |
| 5165 | 47.175 | -121.101 | 815 | 5.81 |
| 5166 | 47.543 | -121.091 | 1056 | 2.08 |
| 5167 | 47.150 | -121.208 | 1008 | 4.16 |
| 5168 | 47.425 | -120.945 | 1280 | 1.92 |
| 5169 | 46.918 | -121.134 | 1264 | 5.19 |
| 5170 | 46.886 | -121.093 | 1242 | 5.69 |

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| 5171 | 46.688 | -120.979 | 1334 | 6.20 |
| 5173 | 46.957 | -120.971 | 1578 | 3.99 |
| 5175 | 46.942 | -121.200 | 987 | 5.23 |
| 5176 | 47.063 | -121.195 | 1076 | 5.25 |
| 5177 | 46.658 | -121.269 | 1014 | 4.93 |
| 5178 | 46.567 | -121.248 | 1097 | 4.63 |
| 5179 | 46.628 | -121.313 | 1197 | 5.01 |
| 5180 | 47.035 | -121.291 | 1511 | 4.35 |
| 5181 | 47.033 | -121.299 | 1597 | 4.35 |
| 5182 | 46.508 | -121.265 | 1313 | 4.16 |
| 5183 | 46.775 | -121.110 | 1211 | 5.40 |
| 5206 | 47.183 | -120.543 | 1460 | 3.46 |
| 6004 | 42.075 | -123.558 | 948 | 11.13 |
| 6005 | 42.075 | -123.548 | 975 | 11.13 |
| 6007 | 42.234 | -123.778 | 1258 | 10.59 |
| 6008 | 42.162 | -123.446 | 1161 | 10.26 |
| 6010 | 42.235 | -123.779 | 1300 | 10.59 |
| 6011 | 42.432 | -124.157 | 745 | 11.68 |
| 6012 | 45.089 | -123.746 | 244 | 10.54 |
| 6013 | 45.194 | -123.672 | 501 | 10.16 |
| 6015 | 45.182 | -123.859 | 396 | 10.80 |
| 6016 | 44.403 | -123.837 | 241 | 11.77 |
| 6017 | 44.169 | -124.054 | 113 | 11.27 |
| 6018 | 44.419 | -123.815 | 208 | 11.58 |
| 6021 | 44.405 | -123.836 | 240 | 11.77 |
| 6023 | 44.396 | -123.778 | 549 | 11.91 |
| 6024 | 44.160 | -123.730 | 337 | 11.96 |
| 6025 | 44.158 | -123.732 | 296 | 11.96 |
| 6026 | 42.506 | -124.266 | 120 | 12.82 |
| 6028 | 42.659 | -124.283 | 605 | 11.90 |
| 6030 | 42.518 | -124.129 | 575 | 12.45 |
| 6031 | 42.464 | -124.278 | 636 | 12.83 |
| 6032 | 42.254 | -124.133 | 251 | 11.78 |
| 6034 | 42.020 | -124.109 | 61 | 12.02 |
| 6035 | 42.315 | -124.294 | 610 | 12.64 |
| 6038 | 42.152 | -124.119 | 486 | 11.68 |
| 6039 | 42.111 | -124.059 | 460 | 11.39 |
| 6040 | 42.050 | -124.021 | 657 | 11.73 |
| 6041 | 42.272 | -124.201 | 852 | 11.66 |
| 6042 | 42.186 | -124.007 | 963 | 11.21 |
| 6047 | 42.713 | -124.385 | 178 | 12.33 |

| | | | | |
|---|---|---|---|---|
| 6050 | 42.811 | -124.295 | 121 | 11.42 |
| 6052 | 42.720 | -124.040 | 358 | 11.65 |
| 6053 | 42.687 | -124.230 | 768 | 11.71 |
| 6054 | 42.779 | -124.209 | 724 | 11.65 |
| 6055 | 42.707 | -124.163 | 792 | 11.09 |
| 6056 | 42.885 | -123.879 | 991 | 10.32 |
| 6057 | 42.864 | -123.977 | 912 | 11.21 |
| 6070 | 42.384 | -123.705 | 916 | 10.72 |
| 6071 | 42.122 | -123.367 | 1194 | 8.59 |
| 6073 | 42.490 | -123.715 | 1227 | 10.47 |
| 6074 | 42.417 | -123.626 | 1113 | 10.49 |
| 6075 | 42.428 | -123.778 | 1072 | 10.86 |
| 6076 | 42.022 | -123.494 | 1284 | 9.41 |
| 6078 | 42.528 | -124.019 | 524 | 13.04 |
| 6079 | 42.691 | -123.875 | 812 | 12.07 |
| 6080 | 42.640 | -123.973 | 726 | 12.08 |
| 6083 | 42.543 | -123.845 | 1007 | 10.87 |
| 6084 | 42.649 | -123.833 | 1084 | 10.46 |
| 6085 | 42.373 | -124.177 | 1075 | 11.51 |
| 6087 | 45.120 | -123.845 | 313 | 10.80 |
| 6088 | 45.310 | -123.887 | 122 | 10.80 |
| 6089 | 45.337 | -123.792 | 278 | 10.70 |
| 6090 | 44.895 | -123.894 | 186 | 10.59 |
| 6091 | 44.969 | -123.860 | 363 | 10.39 |
| 6092 | 44.529 | -123.937 | 120 | 11.36 |
| 6093 | 44.287 | -124.009 | 118 | 11.41 |
| 6094 | 44.387 | -123.980 | 177 | 11.37 |
| 6095 | 44.121 | -124.075 | 243 | 11.10 |
| 6097 | 44.229 | -124.082 | 238 | 11.36 |
| 6099 | 44.294 | -123.799 | 184 | 12.22 |
| 6101 | 44.548 | -123.888 | 426 | 11.44 |
| 6102 | 44.487 | -123.960 | 220 | 11.34 |
| 6104 | 44.262 | -123.880 | 330 | 12.00 |
| 6105 | 44.518 | -123.762 | 441 | 11.33 |
| 6106 | 44.348 | -123.752 | 415 | 12.22 |
| 6107 | 44.118 | -124.024 | 469 | 11.17 |
| 6109 | 44.264 | -123.709 | 346 | 11.87 |
| 6110 | 44.459 | -123.846 | 645 | 11.07 |
| 6113 | 43.979 | -123.933 | 257 | 11.51 |
| 6115 | 44.214 | -123.923 | 407 | 11.79 |
| 6118 | 43.731 | -123.952 | 274 | 11.44 |

| | | | | |
|---|---|---|---|---|
| 6119 | 43.725 | -123.901 | 336 | 11.42 |
| 7201 | 47.098 | -123.855 | 61 | 10.38 |
| 7204 | 47.176 | -123.868 | 61 | 10.24 |
| 7205 | 47.319 | -123.908 | 151 | 9.94 |
| 7208 | 47.238 | -123.836 | 127 | 9.78 |
| 7210 | 47.281 | -123.880 | 122 | 9.87 |
| 7211 | 47.326 | -123.790 | 171 | 9.08 |
| 7213 | 46.633 | -123.669 | 63 | 10.61 |
| 7214 | 46.614 | -123.649 | 61 | 10.48 |
| 7215 | 47.208 | -123.942 | 65 | 10.28 |
| 8003 | 45.138 | -123.758 | 603 | 10.25 |
| 8004 | 45.150 | -123.750 | 586 | 10.25 |
| 8005 | 44.542 | -121.638 | 930 | 7.93 |
| 8007 | 45.540 | -122.338 | 61 | 11.78 |
| 8008 | 45.580 | -122.101 | 61 | 10.26 |
| 8009 | 45.627 | -121.967 | 55 | 10.75 |
| 8010 | 45.626 | -121.969 | 56 | 10.75 |
| 8014 | 45.701 | -121.621 | 61 | 10.09 |
| 8015 | 45.687 | -121.434 | 61 | 10.15 |
| 8016 | 45.686 | -121.432 | 61 | 10.15 |
| 8017 | 45.619 | -121.345 | 606 | 9.98 |
| 8019 | 46.559 | -123.227 | 197 | 10.57 |
| 8020 | 46.558 | -123.227 | 217 | 10.57 |
| 8021 | 47.994 | -123.213 | 647 | 8.20 |
| 8022 | 47.995 | -123.214 | 632 | 8.20 |
| 8023 | 48.039 | -123.053 | 140 | 9.78 |
| 8024 | 48.038 | -123.052 | 141 | 9.78 |
| 8025 | 46.484 | -123.562 | 438 | 9.07 |
| 8026 | 46.490 | -123.569 | 505 | 9.07 |
| 8027 | 46.780 | -123.552 | 162 | 10.21 |
| 8028 | 46.780 | -123.550 | 153 | 10.21 |
| 8029 | 45.224 | -123.341 | 331 | 10.62 |
| 8032 | 44.425 | -121.744 | 1153 | 7.13 |
| 8033 | 44.423 | -121.825 | 1407 | 5.19 |
| 8035 | 48.014 | -123.943 | 555 | 7.54 |
| 8041 | 48.589 | -120.480 | 795 | 6.00 |
| 8043 | 48.598 | -120.571 | 1148 | 4.34 |
| 8044 | 48.599 | -120.563 | 1115 | 4.34 |
| 8045 | 44.672 | -123.749 | 61 | 10.89 |
| 8046 | 44.670 | -123.747 | 61 | 10.89 |
| 8048 | 47.661 | -120.522 | 836 | 7.68 |

| | | | | |
|---|---|---|---|---|
| 8049 | 47.681 | -120.588 | 960 | 8.08 |
| 8053 | 44.511 | -123.560 | 1054 | 10.04 |
| 8060 | 44.676 | -123.543 | 244 | 10.32 |
| 8061 | 44.478 | -123.431 | 183 | 10.84 |
| 8063 | 43.281 | -123.460 | 179 | 12.45 |
| 8067 | 44.358 | -124.006 | 314 | 11.38 |
| 8068 | 44.356 | -124.001 | 314 | 11.38 |
| 8069 | 44.388 | -123.636 | 177 | 11.38 |
| 8070 | 44.386 | -123.636 | 141 | 11.38 |
| 8072 | 44.707 | -123.314 | 176 | 10.64 |
| 8074 | 43.233 | -124.017 | 122 | 11.96 |
| 8075 | 43.181 | -123.741 | 610 | 11.01 |
| 8076 | 43.179 | -123.741 | 610 | 11.01 |
| 8079 | 47.619 | -120.647 | 426 | 8.55 |
| 8080 | 47.617 | -120.648 | 426 | 8.55 |
| 8083 | 44.663 | -123.931 | 61 | 10.61 |
| 8084 | 48.013 | -123.944 | 587 | 7.54 |
| 8086 | 45.819 | -123.002 | 232 | 8.93 |
| 8091 | 44.471 | -123.505 | 442 | 10.50 |
| 8092 | 44.504 | -123.565 | 956 | 9.95 |
| 8098 | 45.945 | -120.916 | 785 | 7.55 |
| 8099 | 45.945 | -120.914 | 780 | 7.55 |
| 8100 | 46.560 | -121.696 | 563 | 8.32 |
| 8503 | 45.253 | -122.309 | 304 | 11.06 |
| 8504 | 45.267 | -122.317 | 125 | 11.06 |
| 8518 | 48.055 | -123.594 | 128 | 8.81 |
| 8519 | 48.056 | -123.595 | 132 | 8.81 |
| 8520 | 48.112 | -122.808 | 89 | 10.39 |
| 8521 | 48.113 | -122.808 | 86 | 10.39 |
| 8522 | 43.200 | -123.350 | 162 | 12.09 |
| 8524 | 44.850 | -123.670 | 381 | 9.27 |
| 8525 | 44.849 | -123.669 | 357 | 9.27 |
| 8526 | 45.870 | -123.180 | 192 | 9.55 |
| 8532 | 45.221 | -123.898 | 91 | 10.81 |
| 8533 | 47.420 | -123.220 | 199 | 10.04 |
| 8534 | 47.420 | -123.220 | 199 | 10.04 |
| 8538 | 46.900 | -122.033 | 547 | 7.57 |
| 8539 | 43.600 | -123.580 | 183 | 12.28 |
| 8541 | 47.000 | -123.400 | 61 | 10.75 |
| 8542 | 47.000 | -123.400 | 61 | 10.75 |
| 8543 | 47.910 | -124.370 | 227 | 9.90 |

| | | | | |
|---|---|---|---|---|
| 8544 | 47.910 | -124.370 | 227 | 9.90 |
| 8545 | 48.880 | -121.950 | 444 | 7.34 |
| 8546 | 48.880 | -121.950 | 444 | 7.34 |
| 8548 | 46.370 | -122.620 | 242 | 9.81 |
| 8552 | 42.860 | -124.057 | 122 | 11.83 |
| 8800 | 48.073 | -122.091 | 112 | 10.35 |
| 8801 | 48.150 | -122.114 | 127 | 10.35 |
| 8811 | 47.084 | -122.672 | 66 | 10.41 |
| 8817 | 48.082 | -121.966 | 128 | 10.33 |
| 8836 | 45.001 | -123.362 | 105 | 10.96 |
| 8847 | 46.832 | -122.859 | 122 | 10.50 |

[a]ANNAVT = Annual average temperature

**TABLE S2**

**Factor loadings for the 17 phenotypic traits used to construct principal components**

| Trait | PC1 | PC2 | PC3 | PC4 |
|---|---|---|---|---|
| budcold | 0.211 | -0.384 | 0.222 | 0.129 |
| ndlcold | 0.246 | -0.296 | 0.318 | 0.088 |
| stmcold | 0.225 | -0.373 | 0.285 | 0.105 |
| BB2 | 0.187 | -0.035 | -0.406 | -0.008 |
| BS1 | 0.293 | -0.236 | -0.106 | -0.005 |
| BS2 | 0.238 | -0.266 | -0.174 | 0.205 |
| DIAM | 0.314 | 0.188 | 0.145 | -0.153 |
| EMEAN | -0.169 | 0.239 | 0.382 | -0.151 |
| FLUSH | 0.085 | 0.345 | 0.089 | 0.609 |
| FLUSHLG | 0.142 | 0.322 | 0.086 | 0.582 |
| HT1 | 0.292 | 0.173 | 0.145 | -0.210 |
| HTINC | 0.308 | 0.158 | -0.232 | -0.077 |
| RTLG | 0.190 | 0.222 | 0.180 | -0.209 |
| RTSH | -0.271 | -0.057 | 0.104 | -0.047 |
| RTWT | 0.295 | 0.200 | 0.140 | -0.220 |
| SHWT | 0.328 | 0.167 | 0.064 | -0.148 |
| TAPER | -0.165 | -0.079 | 0.487 | 0.034 |

**TABLE S3**

**A description of the 117 unique candidate genes from which 384 single nucleotide polymorphisms (SNPs) were chosen for genotyping via Illumina's GoldenGate™ high-throughput platform**

| Locus ID | Gene Product | Attempted[a] | Successful[b] | Source |
|---|---|---|---|---|
| *40s* | 40S ribosomal protein S3a | 3 | 1 | KRUTOVSKY AND NEALE (2005) |
| *4CL1* | 4-coumarate:CoA ligase 1 | 5 | 4 | KRUTOVSKY AND NEALE (2005) |
| *4CL2* | 4-coumarate:CoA ligase 2 | 4 | 3 | KRUTOVSKY AND NEALE (2005) |
| *60s RPL31a* | 60S ribosomal protein L31a | 4 | 3 | KRUTOVSKY AND NEALE (2005) |
| *aba* | abscisic acid inducible protein | 6 | 5 | KRUTOVSKY AND NEALE (2005) |
| *apx* | ascorbate peroxidase | 6 | 5 | KRUTOVSKY AND NEALE (2005) |
| *at1* | α-tubulin | 8 | 5 | KRUTOVSKY AND NEALE (2005) |
| *CD028057.1* | calcium-dependent protein kinase | 1 | 1 | ECKERT *et al.* (2009a) |
| *CN634517.1* | luminal binding protein | 4 | 2 | ECKERT *et al.* (2009a) |
| *CN634677.1* | LRR receptor-like protein kinase | 1 | 1 | ECKERT *et al.* (2009a) |
| *CN634994.1* | ADP-ribosylation factor | 6 | 1 | unpublished |
| *CN635490.1* | rare cold inducible protein | 1 | 1 | ECKERT *et al.* (2009a) |
| *CN635596.1* | phosphate-responsive protein | 3 | 1 | ECKERT *et al.* (2009a) |
| *CN635674.1* | pentatricopeptide (PPR) containing protein | 2 | 1 | ECKERT *et al.* (2009a) |
| *CN635691.1* | homeodomain protein (HB2) | 2 | 2 | ECKERT *et al.* (2009a) |
| *CN636014.1* | heat shock protein 70 kDa | 4 | 0 | ECKERT *et al.* (2009a) |
| *CN636134.1* | CBL-interacting protein kinase | 1 | 0 | unpublished |
| *CN636149.1* | cinnamyl alcohol dehydrogenase | 1 | 0 | ECKERT *et al.* (2009a) |
| *CN636303.1* | actin depolymerizing factor | 1 | 1 | ECKERT *et al.* (2009a) |
| *CN636471.1* | phenylalanine ammonia-lyase | 5 | 5 | ECKERT *et al.* (2009a) |
| *CN636784.1* | S-adenosylmethionine synthetase | 4 | 0 | ECKERT *et al.* (2009a) |
| *CN636795.1* | xyloglucan:xyloglucosyl transferase | 3 | 2 | ECKERT *et al.* (2009a) |
| *CN637244.1* | cysteine protease inhibitor | 2 | 2 | ECKERT *et al.* (2009a) |

| | | | | |
|---|---|---|---|---|
| *CN637306.1* | MYB-like transcription factor | 3 | 3 | Eckert *et al.* (2009a) |
| *CN637339.1* | unknown hypothetical protein | 2 | 2 | Eckert *et al.* (2009a) |
| *CN637473.1* | protein kinase domain containing protein | 1 | 0 | unpublished |
| *CN637587.1* | glycosyl hydrolase family protein | 1 | 0 | unpublished |
| *CN637910.1* | ABC family protein | 3 | 2 | Eckert *et al.* (2009a) |
| *CN637944.1* | bet v I domain containing protein | 1 | 0 | Eckert *et al.* (2009a) |
| *CN638310.1* | chloroplastic copper/zinc-superoxide dismutase | 1 | 0 | unpublished |
| *CN638367.1* | ATP-dependent RNA helicase-like protein | 7 | 2 | Eckert *et al.* (2009a) |
| *CN638381.1* | ABC transporter | 4 | 2 | Eckert *et al.* (2009a) |
| *CN638489.1* | α-expansin | 3 | 3 | Eckert *et al.* (2009a) |
| *CN638545.1* | trans-cinnamate 4-hydroxylase | 5 | 1 | Eckert *et al.* (2009a) |
| *CN638556.1* | transcription regulation protein | 2 | 2 | Eckert *et al.* (2009a) |
| *CN639074.1* | S-adenosylmethionine synthetase | 3 | 2 | Eckert *et al.* (2009a) |
| *CN639087.1* | LRR receptor-like protein kinase | 1 | 1 | Eckert *et al.* (2009a) |
| *CN639211.1* | eukaryotic initiation factor 4A | 1 | 0 | unpublished |
| *CN639236.1* | guanine nucleotide-binding beta subunit protein | 7 | 2 | Eckert *et al.* (2009a) |
| *CN639480.1* | 2-hydroxyacid dehydrogenase | 2 | 2 | Eckert *et al.* (2009a) |
| *CN639782.1* | serine/threonine protein kinase | 4 | 0 | unpublished |
| *CN640010.1* | eukaryotic initiation factor-5 | 4 | 0 | unpublished |
| *CN640155.1* | bicoid-interacting 3 domain containing protein | 2 | 1 | Eckert *et al.* (2009a) |
| *CN640361.1* | zinc-finger (C2H2 type) family protein | 3 | 3 | Eckert *et al.* (2009a) |
| *CN640485.1* | HNH endonuclease domain containing protein | 3 | 3 | Eckert *et al.* (2009a) |
| *CN640521.1* | DNA-binding bromodomain-containing protein | 4 | 4 | Eckert *et al.* (2009a) |
| *CN640694.1* | heat shock cognate protein 70 kDa | 5 | 3 | unpublished |
| *CN641217.1* | somatic embryogenesis receptor-like kinase | 1 | 0 | unpublished |
| *CN641226.1* | LRR receptor-like protein kinase | 1 | 1 | Eckert *et al.* (2009a) |
| *efla* | translation elongation factor-1 | 3 | 0 | Krutovsky and Neale (2005) |
| *erd15* | early response to dehydration protein | 4 | 4 | Krutovsky and Neale (2005) |

| | | | | |
|---|---|---|---|---|
| *ES418315.1* | flavanone 3-hydroxylase | 2 | 0 | Eckert *et al.* (2009a) |
| *ES419198.1* | LIM domain protein | 2 | 1 | Eckert *et al.* (2009a) |
| *ES419223.1* | phytosulfokine precursor | 4 | 2 | Eckert *et al.* (2009a) |
| *ES419242.1* | response regulator protein | 3 | 1 | Eckert *et al.* (2009a) |
| *ES419657.1* | calmodulin | 3 | 2 | Eckert *et al.* (2009a) |
| *ES419739.1* | proline-rich protein | 1 | 0 | unpublished |
| *ES420071.1* | desaturase-like protein | 2 | 1 | unpublished |
| *ES420250.1* | dehydrin-like protein | 12 | 4 | Eckert *et al.* (2009a) |
| *ES420560.1* | HVA22F like protein | 6 | 1 | unpublished |
| *ES420603.1* | dehydrin-like protein | 8 | 3 | Eckert *et al.* (2009a) |
| *ES420757.1* | unknown hypothetical protein | 9 | 5 | Eckert *et al.* (2009a) |
| *ES420771.1* | anaphase promoting complex/cyclsome protein | 2 | 2 | Eckert *et al.* (2009a) |
| *ES420802.1* | MADS-box transcription factor | 1 | 0 | unpublished |
| *ES420862.1* | late embryo abundance (LEA) protein | 3 | 3 | Eckert *et al.* (2009a) |
| *ES421219.1* | UDP-glucosyltransferase family protein | 2 | 2 | Eckert *et al.* (2009a) |
| *ES421311.1* | unknown hypothetical protein | 10 | 5 | Eckert *et al.* (2009a) |
| *ES421603.1* | heat shock protein 90 kDa | 4 | 2 | Eckert *et al.* (2009a) |
| *ES422367.1* | ferritin | 4 | 3 | Eckert *et al.* (2009a) |
| *ES424016.1* | glutathione S-transferase | 5 | 4 | Eckert *et al.* (2009a) |
| *ES425204.1* | 2-phospho-D-glycerate hydroxylase | 2 | 0 | unpublished |
| *ES428620.1* | 14-3-3 protein | 1 | 1 | Eckert *et al.* (2009a) |
| *f3h1* | flavenone-3-hydroxylase | 3 | 3 | Krutovsky and Neale (2005) |
| *f3h2* | flavenone-3-hydroxylase | 4 | 4 | Krutovsky and Neale (2005) |
| *formin* | formin-like protein AHF1 | 3 | 2 | Krutovsky and Neale (2005) |
| *LEA-EMB11* | late embryogenesis abundant EMB11 like protein | 10 | 8 | Krutovsky and Neale (2005) |
| *lp3* | water deficit-inducible protein | 2 | 2 | Krutovsky and Neale (2005) |
| *mt* | metallothionein-like protein | 5 | 1 | Krutovsky and Neale (2005) |
| *Pm_CL135Contig1* | cysteine proteinase | 4 | 3 | Eckert *et al.* (2009a) |

| | | | | |
|---|---|---|---|---|
| *Pm_CL1400Contig1* | alpha-L-arabinofuranosidase/beta-D-xylosidase | 1 | 1 | Eckert *et al.* (2009a) |
| *Pm_CL1692Contig1* | zinc-finger containing protein | 3 | 2 | Eckert *et al.* (2009a) |
| *Pm_CL1811contig1* | chromatin remodeling ATPase | 3 | 3 | Eckert *et al.* (2009a) |
| *Pm_CL1814Contig1* | tetraspanin | 1 | 1 | Eckert *et al.* (2009a) |
| *Pm_CL1868Contig1* | actin depolymerizing factor | 1 | 1 | Eckert *et al.* (2009a) |
| *Pm_CL1994Contig1* | caffeate O-methyltransferase | 9 | 3 | Eckert *et al.* (2009a) |
| *Pm_CL1997Contig1* | sucrose synthase | 1 | 0 | Eckert *et al.* (2009a) |
| *Pm_CL2089Contig1* | putative formide amidohydrolase | 1 | 1 | Eckert *et al.* (2009a) |
| *Pm_CL2133Contig1* | mitochondrial transcription termination factor | 3 | 3 | Eckert *et al.* (2009a) |
| *Pm_CL234Contig1* | rab GTPase | 3 | 3 | Eckert *et al.* (2009a) |
| *Pm_CL61Contig1* | cyclophilin | 4 | 3 | Eckert *et al.* (2009a) |
| *Pm_CL73Contig1* | glycosyl hydrolase family protein | 2 | 2 | Eckert *et al.* (2009a) |
| *Pm_CL783Contig1* | SOUL heme-binding family protein | 4 | 3 | Eckert *et al.* (2009a) |
| *Pm_CL795Contig1* | WD-40 repeat family protein | 1 | 0 | Eckert *et al.* (2009a) |
| *Pm_CL855Contig1* | flavanone 3-hydroxylase | 2 | 1 | Eckert *et al.* (2009a) |
| *Pm_CL919Contig1* | HVA22-like protein | 1 | 1 | Eckert *et al.* (2009a) |
| *Pm_CL922Contig1* | thaumatin-like protein | 2 | 2 | Eckert *et al.* (2009a) |
| *Pm_CL939Contig1* | aluminum-induced protein | 4 | 3 | Eckert *et al.* (2009a) |
| *Pm_CL969Contig1* | cell division cycle protein | 2 | 2 | Eckert *et al.* (2009a) |
| *sM13Df243* | arabinogalactan 4 | 1 | 0 | Eckert *et al.* (2009a) |
| *sSPcDFD005F06506* | regulator of chromosome condensation protein | 2 | 2 | Eckert *et al.* (2009a) |
| *sSPcDFD024D11311* | polcalcin | 2 | 2 | Eckert *et al.* (2009a) |
| *sSPcDFD040B03103* | MADS-box transcription factor | 3 | 3 | Eckert *et al.* (2009a) |
| *sSPcDFE002A03003* | ACC oxidase | 3 | 1 | Eckert *et al.* (2009a) |
| *sSPcDFE003F04504* | ccr4-NOT transcription complex protein | 4 | 2 | Eckert *et al.* (2009a) |
| *sSPcDFE025C06206* | purple acid phosphatase | 2 | 2 | Eckert *et al.* (2009a) |
| *sSPcDFE028B10110* | β-amylase | 4 | 4 | Eckert *et al.* (2009a) |
| *sSPcDFE038D06306* | calcium binding protein with EF-hand motif | 3 | 2 | Eckert *et al.* (2009a) |
| *sSPcDFE044F10510* | mitochondrial substrate carrier family protein | 4 | 3 | Eckert *et al.* (2009a) |
| *sSPcDFE049B06106* | auxin-responsive family protein | 1 | 1 | Eckert *et al.* (2009a) |

| | | | | |
|---|---|---|---|---|
| *sSPcDFE049E11411* | pentatricopeptide (PPR) containing protein | 4 | 4 | ECKERT *et al.* (2009a) |
| *sSPcDFF014F08508* | hypothetical water stress induced protein | 1 | 1 | ECKERT *et al.* (2009a) |
| *sSPcDFF015H05705* | cytochrome P450 family protein | 4 | 4 | ECKERT *et al.* (2009a) |
| *sSPcDFF020H04704* | cytochrome P450 mono-oxygenase | 1 | 0 | unpublished |
| *sSPcDFF044H10710* | auxin:hydrogen symporter/transporter | 4 | 2 | ECKERT *et al.* (2009a) |
| *tbe* | thiazole biosynthetic enzyme | 5 | 3 | KRUTOVSKY AND NEALE (2005) |
| *ubq* | polyubiquitin | 11 | 3 | KRUTOVSKY AND NEALE (2005) |
| *Z49715.1* | late embryo abundance (LEA) protein | 2 | 0 | ECKERT *et al.* (2009a) |
| Total | 117 candidate genes | 384 (3 ± 2) | 228 (2 ± 1) | ------ |

[a]The number of SNPs selected for genotyping using the GoldenGate™ platform. Counts greater than those reported in ECKERT *et al.* (2009a) were obtained from further sequencing of overlapping gene fragments. In all cases, only those SNPs reported by ECKERT *et al.* (2009a) produced successful genotyping results. Unpublished data are from the initial candidate gene search and sequencing efforts. The data and alignment quality, as well as the sample sizes ($n < 6$) were marginal and thus not reported by ECKERT *et al.* (2009a). These data were included as part of the effort to maximize the number of genes during our genotyping efforts.

[b]The number of attempted SNPs successfully genotyped using thresholds of 0.35 and 0.85 for the $\text{GenCall}_{50}$ ($\text{GC}_{50}$) and call rate (CR) indices, respectively (cf. Materials and Methods).

**TABLE S4**

**A summary of trait distributions for families located on the east (*n* = 57) or west (*n* = 643) side of the Cascade crest in northeastern Washington**

| | Mean | | SD | | 2.5% percentile | | 97.5% percentile | | |
|---|---|---|---|---|---|---|---|---|---|
| Trait | West | East | West | East | West | East | West | East | *P* |
| Emergence | | | | | | | | | |
| EMEAN | 0.0464 | 0.0548 | 0.0036 | 0.0052 | 0.0398 | 0.0437 | 0.0537 | 0.0626 | <0.0001 |
| EMSTD | 0.0040 | 0.0051 | 0.0008 | 0.0013 | 0.0026 | 0.0030 | 0.0058 | 0.0071 | <0.0001 |
| Growth and Resource Partitioning | | | | | | | | | |
| DIAM | 6.2831 | 4.6459 | 0.5905 | 0.8913 | 5.1563 | 3.0689 | 7.4896 | 6.1719 | <0.0001 |
| FLUSH | 0.3663 | 0.1858 | 0.1480 | 0.1335 | 0.1027 | 0.0435 | 0.6658 | 0.5448 | <0.0001 |
| FLUSHLG | 3.0244 | 1.2815 | 1.3574 | 1.0945 | 0.7679 | 0.1349 | 6.0065 | 4.1533 | <0.0001 |
| HT1 | 12.7592 | 9.8549 | 1.4007 | 2.0557 | 10.1291 | 5.9187 | 15.4611 | 13.2288 | <0.0001 |
| HT2 | 34.8334 | 22.5848 | 4.2208 | 5.8961 | 26.8076 | 11.0347 | 43.0884 | 31.3365 | <0.0001 |
| HTINC | 22.1169 | 13.3210 | 3.3813 | 3.5290 | 15.6379 | 6.0605 | 28.9628 | 18.6697 | <0.0001 |
| RTLG | 34.0180 | 30.4717 | 2.3974 | 3.3505 | 29.8599 | 25.1587 | 38.8477 | 36.5531 | <0.0001 |
| RTSH | 0.4057 | 0.5061 | 0.0488 | 0.0791 | 0.3274 | 0.4075 | 0.5275 | 0.6784 | <0.0001 |
| RTWT | 3.4341 | 1.9156 | 0.6514 | 0.7266 | 2.3390 | 0.6280 | 4.7303 | 3.3014 | <0.0001 |
| SHWT | 9.1297 | 3.4978 | 2.1257 | 2.2642 | 5.3797 | -0.5000 | 13.9938 | 7.0767 | <0.0001 |
| TAPER | 0.1868 | 0.2018 | 0.0154 | 0.0153 | 0.1598 | 0.1808 | 0.2203 | 0.2377 | <0.0001 |
| TOTWT | 12.5620 | 5.4211 | 2.7149 | 2.9884 | 7.8000 | 0.1010 | 18.2476 | 10.4156 | <0.0001 |
| Phenology and Cold-Tolerance | | | | | | | | | |
| BB2 | 106.4323 | 93.7657 | 4.3771 | 4.5461 | 98.5273 | 85.0478 | 115.5121 | 101.2728 | <0.0001 |
| BS1 | 273.8946 | 251.9865 | 7.1460 | 6.7834 | 257.7470 | 237.2549 | 287.2101 | 262.2807 | <0.0001 |
| BS2 | 223.6687 | 197.2985 | 11.7032 | 5.8762 | 199.2543 | 189.6081 | 245.0801 | 213.3060 | <0.0001 |
| budcold | 4.4452 | 2.4618 | 1.2943 | 0.6507 | 2.0945 | 1.3837 | 6.8559 | 3.6764 | <0.0001 |
| ndlcold | 4.5273 | 0.8294 | 2.0766 | 0.4378 | 0.9820 | 0.1657 | 8.6796 | 1.6854 | <0.0001 |
| stmcold | 2.3840 | 0.0804 | 1.4293 | 0.2210 | 0.2351 | -0.1834 | 5.5948 | 0.5261 | <0.0001 |
| Other | | | | | | | | | |
| SDWT | 0.6006 | -6.6191 | 1.8811 | 2.3952 | -3.6039 | -12.0721 | 3.6660 | -3.4418 | 0.0094 |
| Multivariate Traits | | | | | | | | | |
| Prin1 | 0.6006 | -6.6191 | 1.8811 | 2.3952 | -3.6039 | -12.0721 | 3.6660 | -3.4418 | <0.0001 |
| Prin2 | -0.0248 | 0.2633 | 1.6444 | 1.3845 | -3.3419 | -1.9155 | 2.7882 | 2.7883 | 0.2577 |
| Prin3 | -0.0607 | 0.7208 | 1.4232 | 0.9262 | -2.6961 | -0.8909 | 2.8324 | 2.5884 | <0.0001 |
| Prin4 | 0.0561 | -0.6562 | 1.2193 | 1.2480 | -2.2766 | -2.7710 | 2.4270 | 1.7803 | 0.0003 |

**TABLE S5**

**A list of significant marker-trait associations (FDR $Q \leq 0.10$) for the complete data set when the 57 families located east of the Cascade crest are included ($n = 700$)**

| Trait | Locus | Gene Product | SNP | AS[a] | Annotation[b] | *F* | *P* | *Q* | $r^2$ |
|---|---|---|---|---|---|---|---|---|---|
| Emergence | | | | | | | | | |
| EMEAN | *60s RPL31a*-418 | 60s RPL31a | [A/G] | --- | Syn | 6.3376 | 0.0019 | 0.0731 | 0.0162 |
| EMEAN | *ES421311.1*-369 | unknown hypothetical protein | [A/G] | G | NC | 9.8506 | 0.0018 | 0.0709 | 0.0114 |
| EMEAN | *Pm_CL61Contig1*-134 | cyclophilin | [A/G] | A | NC | 5.7559 | 0.0033 | 0.0941 | 0.0134 |
| EMEAN | *Pm_CL783Contig1*-212 | SOUL heme-binding family protein | [A/G] | G | NS | 14.3703 | 0.0002 | 0.0165 | 0.0164 |
| EMSTD | *ES421311.1*-369 | unknown hypothetical protein | [A/G] | G | NC | 15.1370 | 0.0001 | 0.0130 | 0.0198 |
| EMSTD | *Pm_CL2089Contig1*-164 | formide amidohydrolase | [A/G] | A | NC | 5.7596 | 0.0033 | 0.0941 | 0.0152 |
| EMSTD | *ES424016.1*-304 | glutathione S-transferase | [A/G] | G | Syn | 12.9894 | $2.9 \times 10^{-6}$ | 0.0014 | 0.0335 |
| EMSTD | *sSPcDFE028B10110*-166 | β-amylase | [A/G] | G | Syn | 5.7275 | 0.0034 | 0.0964 | 0.0152 |
| Growth and Resource Partitioning | | | | | | | | | |
| DIAM | *4CL1*-520 | 4-coumarate:CoA ligase 1 | [A/G] | --- | NS | 8.0482 | 0.0004 | 0.0280 | 0.0188 |
| DIAM | *CN636471.1*-406 | phenylalanine ammonia-lyase | [A/G] | G | Syn | 5.8081 | 0.0032 | 0.0923 | 0.0139 |
| DIAM | *CN636471.1*-437 | phenylalanine ammonia-lyase | [C/G] | C | Syn | 5.8401 | 0.0031 | 0.0916 | 0.0140 |
| DIAM | *ES420757.1*-311 | unknown hypothetical protein | [A/C] | C | Syn | 14.1490 | 0.0002 | 0.0182 | 0.0168 |
| DIAM | *Pm_CL135Contig1*-665 | cysteine proteinase | [A/G] | G | NC | 12.2976 | 0.0005 | 0.0350 | 0.0144 |
| DIAM | *Pm_CL61Contig1*-134 | cyclophilin | [A/G] | A | NC | 11.3928 | $1.4 \times 10^{-6}$ | 0.0029 | 0.0263 |
| DIAM | *Pm_CL783Contig1*-212 | SOUL heme-binding family protein | [A/G] | G | NS | 14.1102 | 0.0002 | 0.0182 | 0.0165 |
| DIAM | *Pm_CL919Contig1*-355 | HVA22-like protein | [A/C] | A | NS | 5.9010 | 0.0029 | 0.0888 | 0.0138 |
| DIAM | *ES422367.1*-165 | ferritin | [A/T] | T | NC | 8.5368 | 0.0036 | 0.0987 | 0.0124 |
| DIAM | *CN639236.1*-518 | guanine nucleotide-binding protein | [A/G] | A | Syn | 7.6505 | 0.0005 | 0.0363 | 0.0185 |
| DIAM | *sSPcDFD040B03103*-274 | MADS-box transcription factor | [A/G] | G | Syn | 10.0015 | 0.0016 | 0.0679 | 0.0118 |
| DIAM | *sSPcDFE049E11411*-220 | pentatricopeptide-containing protein | [A/G] | A | NS | 8.2101 | 0.0003 | 0.0256 | 0.0196 |
| HT1 | *4CL1*-520 | 4-coumarate:CoA ligase 1 | [A/G] | --- | NS | 6.9221 | 0.0011 | 0.0569 | 0.0173 |
| HT1 | *Pm_CL61Contig1*-134 | cyclophilin | [A/G] | A | NC | 9.6428 | 0.0001 | 0.0102 | 0.0240 |
| HT1 | *Pm_CL783Contig1*-212 | SOUL heme-binding family protein | [A/G] | G | NS | 13.0680 | 0.0003 | 0.0263 | 0.0164 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| HT1 | *Pm_CL919Contig1*-355 | HVA22-like protein | [A/C] | A | NS | 6.8173 | 0.0012 | 0.0573 | 0.0170 |
| HT2 | *ES420757.1*-311 | unknown hypothetical protein | [A/C] | C | Syn | 10.4425 | 0.0013 | 0.0603 | 0.0123 |
| HT2 | *Pm_CL61Contig1*-134 | cyclophilin | [A/G] | A | NC | 9.0032 | 0.0001 | 0.0149 | 0.0207 |
| HT2 | *Pm_CL783Contig1*-212 | SOUL heme-binding family protein | [A/G] | G | NS | 22.4929 | $2.6x10^{-6}$ | 0.0014 | 0.0256 |
| HT2 | *Pm_CL919Contig1*-355 | HVA22-like protein | [A/C] | A | NS | 5.9738 | 0.0027 | 0.0853 | 0.0138 |
| HT2 | *sSPcDFE049E11411*-220 | pentatricopeptide-containing protein | [A/G] | A | NS | 5.7961 | 0.0032 | 0.0923 | 0.0138 |
| HTINC | *ES420757.1*-311 | unknown hypothetical protein | [A/C] | C | Syn | 9.0177 | 0.0028 | 0.0863 | 0.0109 |
| HTINC | *Pm_CL61Contig1*-134 | cyclophilin | [A/G] | A | NC | 6.1006 | 0.0024 | 0.0816 | 0.0144 |
| HTINC | *Pm_CL783Contig1*-212 | SOUL heme-binding family protein | [A/G] | G | NS | 20.6848 | $6.4x10^{-6}$ | 0.0018 | 0.0241 |
| RTLG | *Pm_CL919Contig1*-355 | HVA22-like protein | [A/C] | A | NS | 5.9251 | 0.0028 | 0.0863 | 0.0154 |
| RTSH | *4CL1*-363 | 4-coumarate:CoA ligase 1 | [A/G] | --- | NS | 9.4384 | 0.0022 | 0.0785 | 0.0118 |
| RTSH | *Pm_CL783Contig1*-212 | SOUL heme-binding family protein | [A/G] | G | NS | 23.4072 | $1.6x10^{-6}$ | 0.0012 | 0.0288 |
| RTWT | *CN636471.1*-406 | phenylalanine ammonia-lyase | [A/G] | G | Syn | 5.8174 | 0.0031 | 0.0916 | 0.0145 |
| RTWT | *CN636471.1*-437 | phenylalanine ammonia-lyase | [C/G] | C | Syn | 6.8054 | 0.0012 | 0.0573 | 0.0169 |
| RTWT | *ES420757.1*-311 | unknown hypothetical protein | [A/C] | C | Syn | 10.1984 | 0.0015 | 0.0647 | 0.0126 |
| RTWT | *Pm_CL135Contig1*-665 | cysteine proteinase | [A/G] | G | NC | 9.4136 | 0.0022 | 0.0785 | 0.0115 |
| RTWT | *Pm_CL61Contig1*-134 | cyclophilin | [A/G] | A | NC | 11.3955 | $1.4x10^{-5}$ | 0.0029 | 0.0274 |
| RTWT | *Pm_CL783Contig1*-212 | SOUL heme-binding family protein | [A/G] | G | NS | 11.0928 | 0.0009 | 0.0515 | 0.0136 |
| RTWT | *Pm_CL919Contig1*-355 | HVA22-like protein | [A/C] | A | NS | 6.8673 | 0.0011 | 0.0569 | 0.0167 |
| RTWT | *sSPcDFE049E11411*-220 | pentatricopeptide-containing protein | [A/G] | A | NS | 9.5940 | 0.0001 | 0.0102 | 0.0237 |
| SHWT | *CN636471.1*-437 | phenylalanine ammonia-lyase | [C/G] | C | Syn | 6.0217 | 0.0026 | 0.0838 | 0.0145 |
| SHWT | *ES420757.1*-311 | unknown hypothetical protein | [A/C] | C | Syn | 10.3524 | 0.0014 | 0.0620 | 0.0125 |
| SHWT | *Pm_CL135Contig1*-665 | cysteine proteinase | [A/G] | G | NC | 10.3984 | 0.0013 | 0.0603 | 0.0124 |
| SHWT | *Pm_CL61Contig1*-134 | cyclophilin | [A/G] | A | NC | 12.5203 | $4.6x10^{-6}$ | 0.0014 | 0.0291 |
| SHWT | *Pm_CL783Contig1*-212 | SOUL heme-binding family protein | [A/G] | G | NS | 19.6600 | $1.1x10^{-5}$ | 0.0027 | 0.0231 |
| SHWT | *Pm_CL919Contig1*-355 | HVA22-like protein | [A/C] | A | NS | 6.1195 | 0.0023 | 0.0809 | 0.0145 |
| SHWT | *sSPcDFE049E11411*-220 | pentatricopeptide-containing protein | [A/G] | A | NS | 8.6715 | 0.0002 | 0.0183 | 0.0209 |
| TAPER | *aba*-609 | abscisic acid inducible protein | [A/G] | --- | NS | 6.0486 | 0.0025 | 0.0821 | 0.0166 |
| TAPER | *LEA-EMB11*-263 | late embryogenesis abundant protein | [A/C] | --- | NC | 13.2628 | 0.0003 | 0.0256 | 0.0185 |
| TAPER | *Pm_CL783Contig1*-212 | SOUL heme-binding family protein | [A/G] | G | NS | 9.8753 | 0.0017 | 0.0691 | 0.0132 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| TOTWT | *CN636471.1*-437 | phenylalanine ammonia-lyase | [C/G] | C | Syn | 6.3720 | 0.0018 | 0.0709 | 0.0154 |
| TOTWT | *ES420757.1*-311 | unknown hypothetical protein | [A/C] | C | Syn | 10.5291 | 0.0012 | 0.0573 | 0.0127 |
| TOTWT | *Pm_CL135Contig1*-665 | cysteine proteinase | [A/G] | G | NC | 10.5538 | 0.0012 | 0.0573 | 0.0126 |
| TOTWT | *Pm_CL61Contig1*-134 | cyclophilin | [A/G] | A | NC | 12.8221 | $3.4x10^{-6}$ | 0.0014 | 0.0298 |
| TOTWT | *Pm_CL783Contig1*-212 | SOUL heme-binding family protein | [A/G] | G | NS | 18.1082 | $2.4x10^{-5}$ | 0.0045 | 0.0213 |
| TOTWT | *Pm_CL919Contig1*-355 | HVA22-like protein | [A/C] | A | NS | 6.4525 | 0.0017 | 0.0691 | 0.0153 |
| TOTWT | *sSPcDFE049E11411*-220 | pentatricopeptide-containing protein | [A/G] | A | NS | 9.0495 | 0.0001 | 0.0147 | 0.0218 |
| Phenology and Cold-Tolerance | | | | | | | | | |
| BB2 | *60s RPL31a*-295 | 60s RPL31a | [A/G] | --- | Syn | 12.3169 | 0.0005 | 0.0350 | 0.0136 |
| BB2 | *60s RPL31a*-418 | 60s RPL31a | [A/G] | --- | Syn | 6.8759 | 0.0011 | 0.0569 | 0.0165 |
| BB2 | *60s RPL31a*-55 | 60s RPL31a | [A/G] | --- | NC | 6.7829 | 0.0012 | 0.0573 | 0.0162 |
| BB2 | *LEA-EMB11*-263 | late embryogenesis abundant protein | [A/C] | --- | NC | 9.6815 | 0.0019 | 0.0731 | 0.0110 |
| BB2 | *ES420757.1*-311 | unknown hypothetical protein | [A/C] | C | Syn | 13.3489 | 0.0003 | 0.0252 | 0.0148 |
| BB2 | *Pm_CL61Contig1*-134 | cyclophilin | [A/G] | A | NC | 6.4892 | 0.0016 | 0.0679 | 0.0143 |
| BS1 | *4CL1*-520 | 4-coumarate:CoA ligase 1 | [A/G] | --- | NS | 6.3054 | 0.0019 | 0.0731 | 0.0147 |
| BS1 | *60s RPL31a*-55 | 60s RPL31a | [A/G] | --- | NC | 7.2394 | 0.0008 | 0.0478 | 0.0185 |
| BS1 | *at1*-329 | α-tubulin | [A/C] | --- | NS | 10.5138 | 0.0012 | 0.0573 | 0.0124 |
| BS1 | *CN637306.1*-520 | MYB-like transcription factor | [A/G] | G | NC | 7.4740 | 0.0006 | 0.0412 | 0.0173 |
| BS1 | *CN641226.1*-250 | LRR receptor-like protein kinase | [A/G] | A | Syn | 5.7111 | 0.0035 | 0.0970 | 0.0137 |
| BS1 | *ES420757.1*-311 | unknown hypothetical protein | [A/C] | C | Syn | 18.9944 | $1.5x10^{-5}$ | 0.0031 | 0.0221 |
| BS1 | *CN636303.1*-403 | actin depolymerizing factor | [A/G] | G | NC | 8.8855 | 0.0030 | 0.0902 | 0.0103 |
| BS1 | *Pm_CL61Contig1*-134 | cyclophilin | [A/G] | A | NC | 14.2304 | $8.8x10^{-7}$ | 0.0009 | 0.0323 |
| BS1 | *Pm_CL783Contig1*-212 | SOUL heme-binding family protein | [A/G] | G | NS | 28.4701 | $1.3x10^{-7}$ | 0.0002 | 0.0323 |
| BS1 | *sSPcDFE049E11411*-125 | pentatricopeptide-containing protein | [A/G] | A | Syn | 7.0908 | 0.0009 | 0.0512 | 0.0171 |
| BS1 | *sSPcDFE049E11411*-220 | pentatricopeptide-containing protein | [A/G] | A | NS | 12.3408 | $5.5x10^{-6}$ | 0.0016 | 0.0289 |
| BS1 | *sSPcDFE049E11411*-306 | pentatricopeptide-containing protein | [A/G] | A | NS | 6.5533 | 0.0015 | 0.0647 | 0.0152 |
| BS2 | *60s RPL31a*-295 | 60s RPL31a | [A/G] | --- | Syn | 8.5796 | 0.0035 | 0.0970 | 0.0109 |
| BS2 | *60s RPL31a*-418 | 60s RPL31a | [A/G] | --- | Syn | 8.5957 | 0.0002 | 0.0192 | 0.0237 |
| BS2 | *60s RPL31a*-55 | 60s RPL31a | [A/G] | --- | NC | 6.5477 | 0.0015 | 0.0647 | 0.0181 |
| BS2 | *CN637306.1*-520 | MYB-like transcription factor | [A/G] | G | NC | 7.0210 | 0.0010 | 0.0535 | 0.0176 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| BS2 | *ES420757.1*-311 | unknown hypothetical protein | [A/C] | C | Syn | 9.2034 | 0.0025 | 0.0821 | 0.0118 |
| BS2 | *CN636303.1*-403 | actin depolymerizing factor | [A/G] | G | NC | 12.2867 | 0.0005 | 0.0350 | 0.0154 |
| BS2 | *Pm_CL61Contig1*-134 | cyclophilin | [A/G] | A | NC | 7.1318 | 0.0009 | 0.0497 | 0.0179 |
| BS2 | *Pm_CL783Contig1*-212 | SOUL heme-binding family protein | [A/G] | G | NS | 25.2455 | $6.5x10^{-7}$ | 0.0008 | 0.0310 |
| BS2 | *sSPcDFE049E11411*-220 | pentatricopeptide-containing protein | [A/G] | A | NS | 7.4465 | 0.0006 | 0.0419 | 0.0192 |
| budcold | *4CL1*-520 | 4-coumarate:CoA ligase 1 | [A/G] | --- | NS | 13.7753 | $1.4x10^{-6}$ | 0.0011 | 0.0362 |
| budcold | *60s RPL31a*-418 | 60s RPL31a | [A/G] | --- | Syn | 5.7003 | 0.0035 | 0.0970 | 0.0171 |
| budcold | *60s RPL31a*-55 | 60s RPL31a | [A/G] | --- | NC | 6.4081 | 0.0018 | 0.0709 | 0.0190 |
| budcold | *CN637306.1*-381 | MYB-like transcription factor | [A/G] | A | Syn | 6.2501 | 0.0020 | 0.0758 | 0.0168 |
| budcold | *CN638489.1*-116 | α-expansin | [A/G] | G | Syn | 5.6775 | 0.0036 | 0.0987 | 0.0157 |
| budcold | *CN640521.1*-370 | DNA-binding bromodomain-containing protein | [A/G] | A | NS | 5.6393 | 0.0037 | 0.0998 | 0.0151 |
| budcold | *erd15*-635 | early response to dehydration protein | [A/C] | --- | NC | 6.8841 | 0.0011 | 0.0569 | 0.0184 |
| budcold | *f3h2*-54 | flavanone-3-hydroxylase | [A/C] | --- | NC | 8.2260 | 0.0003 | 0.0256 | 0.0219 |
| budcold | *ES420757.1*-311 | unknown hypothetical protein | [A/C] | C | Syn | 10.2329 | 0.0014 | 0.0620 | 0.0140 |
| budcold | *Pm_CL1692Contig1*-234 | zinc-finger containing protein | [A/G] | A | Syn | 5.9370 | 0.0028 | 0.0863 | 0.0163 |
| budcold | *Pm_CL234Contig1*-156 | rab GTPase | [A/T] | T | NC | 6.0741 | 0.0024 | 0.0816 | 0.0176 |
| budcold | *Pm_CL61Contig1*-134 | cyclophilin | [A/G] | A | NC | 11.6125 | $3.2x10^{-5}$ | 0.0027 | 0.0308 |
| budcold | *Pm_CL783Contig1*-212 | SOUL heme-binding family protein | [A/G] | G | NS | 15.3136 | 0.0001 | 0.0121 | 0.0204 |
| budcold | *CN637244.1*-220 | cysteine protease inhibitor | [C/G] | C | NC | 6.7158 | 0.0013 | 0.0603 | 0.0194 |
| budcold | *sSPcDFD040B03103*-274 | MADS-box transcription factor | [A/G] | G | Syn | 13.1808 | 0.0003 | 0.0256 | 0.0177 |
| budcold | *sSPcDFE049E11411*-220 | pentatricopeptide-containing protein | [A/G] | A | NS | 7.3727 | 0.0007 | 0.0439 | 0.0204 |
| ndlcold | *4CL1*-520 | 4-coumarate:CoA ligase 1 | [A/G] | --- | NS | 12.9014 | $3.2x10^{-6}$ | 0.0014 | 0.0332 |
| ndlcold | *60s RPL31a*-55 | 60s RPL31a | [A/G] | --- | NC | 6.4611 | 0.0017 | 0.0691 | 0.0189 |
| ndlcold | *CN637306.1*-381 | MYB-like transcription factor | [A/G] | A | Syn | 7.1845 | 0.0008 | 0.0483 | 0.0188 |
| ndlcold | *CN639480.1*-430 | 2-hydroxyacid dehydrogenase | [A/G] | A | Syn | 6.1704 | 0.0022 | 0.0785 | 0.0165 |
| ndlcold | *erd15*-635 | early response to dehydration protein | [A/C] | --- | NC | 8.0377 | 0.0004 | 0.0280 | 0.0209 |
| ndlcold | *f3h2*-54 | flavanone-3-hydroxylase | [A/C] | --- | NC | 10.8717 | $2.3x10^{-5}$ | 0.0045 | 0.0281 |
| ndlcold | *LEA-EMB11*-263 | late embryogenesis abundant protein | [A/C] | --- | NC | 10.3540 | 0.0014 | 0.0620 | 0.0142 |
| ndlcold | *ES420757.1*-311 | unknown hypothetical protein | [A/C] | C | Syn | 14.8376 | 0.0001 | 0.0144 | 0.0197 |
| ndlcold | *Pm_CL135Contig1*-665 | cysteine proteinase | [A/G] | G | NC | 16.2904 | 0.0001 | 0.0086 | 0.0213 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| ndlcold | *Pm_CL234Contig1*-156 | rab GTPase | [A/T] | T | NC | 5.6360 | 0.0037 | 0.0998 | 0.0159 |
| ndlcold | *Pm_CL61Contig1*-134 | cyclophilin | [A/G] | A | NC | 10.5164 | $3.2x10^{-5}$ | 0.0053 | 0.0273 |
| ndlcold | *Pm_CL783Contig1*-212 | SOUL heme-binding family protein | [A/G] | G | NS | 21.6080 | $4.0x10^{-6}$ | 0.0014 | 0.0280 |
| ndlcold | *CN637244.1*-220 | cysteine protease inhibitor | [C/G] | C | NC | 7.7463 | 0.0005 | 0.0350 | 0.0219 |
| ndlcold | *sSPcDFD040B03103*-274 | MADS-box transcription factor | [A/G] | G | Syn | 17.3655 | $3.5x10^{-5}$ | 0.0056 | 0.0227 |
| ndlcold | *sSPcDFE049E11411*-220 | pentatricopeptide-containing protein | [A/G] | A | NS | 6.2044 | 0.0021 | 0.0778 | 0.0168 |
| stmcold | *4CL1*-520 | 4-coumarate:CoA ligase 1 | [A/G] | --- | NS | 11.5068 | $1.2x10^{-5}$ | 0.0029 | 0.0301 |
| stmcold | *4CL2*-459 | 4-coumarate:CoA ligase 1 | [A/C] | --- | NS | 8.7503 | 0.0032 | 0.0923 | 0.0118 |
| stmcold | *60s RPL31a*-55 | 60s RPL31a | [A/G] | --- | NC | 6.2177 | 0.0021 | 0.0778 | 0.0183 |
| stmcold | *CN637306.1*-381 | MYB-like transcription factor | [A/G] | A | Syn | 10.2248 | $4.2x10^{-5}$ | 0.0065 | 0.0269 |
| stmcold | *CN637306.1*-520 | MYB-like transcription factor | [A/G] | G | NC | 6.1704 | 0.0022 | 0.0785 | 0.0163 |
| stmcold | *CN637339.1*-337 | unknown hypothetical protein | [A/G] | A | NS | 7.1478 | 0.0009 | 0.0497 | 0.0202 |
| stmcold | *CN638489*.1-116 | α-expansin | [A/G] | G | Syn | 6.2658 | 0.0020 | 0.0758 | 0.0171 |
| stmcold | *erd15*-635 | early response to dehydration protein | [A/C] | --- | NC | 7.5300 | 0.0006 | 0.0396 | 0.0199 |
| stmcold | *f3h2*-54 | flavanone-3-hydroxylase | [A/C] | --- | NC | 12.5977 | $4.2x10^{-6}$ | 0.0014 | 0.0328 |
| stmcold | *LEA-EMB11*-263 | late embryogenesis abundant protein | [A/C] | --- | NC | 10.2497 | 0.0014 | 0.0620 | 0.0142 |
| stmcold | *ES420757.1*-311 | unknown hypothetical protein | [A/C] | C | Syn | 10.2807 | 0.0014 | 0.0620 | 0.0139 |
| stmcold | *Pm_CL135Contig1*-665 | cysteine proteinase | [A/G] | G | NC | 12.0809 | 0.0005 | 0.0374 | 0.0161 |
| stmcold | *Pm_CL234Contig1*-156 | rab GTPase | [A/T] | T | NC | 7.7964 | 0.0005 | 0.0345 | 0.0221 |
| stmcold | *Pm_CL61Contig1*-134 | cyclophilin | [A/G] | A | NC | 8.9471 | 0.0002 | 0.0154 | 0.0237 |
| stmcold | *Pm_CL783Contig1*-212 | SOUL heme-binding family protein | [A/G] | G | NS | 16.9519 | $4.3x10^{-5}$ | 0.0065 | 0.0223 |
| stmcold | *CN637244.1*-220 | cysteine protease inhibitor | [C/G] | C | NC | 8.0176 | 0.0004 | 0.0283 | 0.0229 |
| stmcold | *sSPcDFD040B03103*-274 | MADS-box transcription factor | [A/G] | G | Syn | 11.6373 | 0.0007 | 0.0439 | 0.0155 |
| stmcold | *sSPcDFE049E11411*-220 | pentatricopeptide-containing protein | [A/G] | A | NS | 6.2044 | 0.0021 | 0.0778 | 0.0170 |
| Other | | | | | | | | | |
| SDWT | *ES420771.1*-88 | anaphase promoting complex protein | [A/C] | A | NS | 9.1071 | 0.0026 | 0.0838 | 0.0127 |
| Multivariate Traits | | | | | | | | | |
| Prin1 | *4CL1*-520 | 4-coumarate:CoA ligase 1 | [A/G] | --- | NS | 9.5863 | 0.0001 | 0.0102 | 0.0207 |
| Prin1 | *at1*-329 | α-tubulin | [A/C] | --- | NS | 9.0435 | 0.0027 | 0.0853 | 0.0100 |
| Prin1 | *CN636471.1*-437 | phenylalanine ammonia-lyase | [C/G] | C | Syn | 6.9327 | 0.0010 | 0.0545 | 0.0154 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Prin1 | *CN637306.1*-520 | MYB-like transcription factor | [A/G] | G | NC | 6.7721 | 0.0012 | 0.0573 | 0.0147 |
| Prin1 | *LEA-EMB11*-372 | late embryogenesis abundant protein | [C/G] | --- | NC | 6.1101 | 0.0023 | 0.0809 | 0.0135 |
| Prin1 | *ES420757.1*-311 | unknown hypothetical protein | [A/C] | C | Syn | 21.4710 | $4.3x10^{-6}$ | 0.0014 | 0.0233 |
| Prin1 | *Pm_CL135Contig1*-665 | cysteine proteinase | [A/G] | G | NC | 14.0041 | 0.0002 | 0.0185 | 0.0153 |
| Prin1 | *Pm_CL61Contig1*-134 | cyclophilin | [A/G] | A | NC | 17.4591 | $4.0x10^{-8}$ | 0.0001 | 0.0368 |
| Prin1 | *Pm_CL783Contig1*-212 | SOUL heme-binding family protein | [A/G] | G | NS | 36.9866 | $2.0x10^{-9}$ | <0.0001 | 0.0390 |
| Prin1 | *Pm_CL919Contig1*-355 | HVA22-like protein | [A/C] | A | NS | 5.8735 | 0.0030 | 0.0902 | 0.0128 |
| Prin1 | *sSPcDFD040B03103*-274 | MADS-box transcription factor | [A/G] | G | Syn | 11.5676 | 0.0007 | 0.0446 | 0.0126 |
| Prin1 | *sSPcDFE049E11411*-125 | pentatricopeptide-containing protein | [A/G] | A | Syn | 6.0511 | 0.0025 | 0.0821 | 0.0137 |
| Prin1 | *sSPcDFE049E11411*-220 | pentatricopeptide-containing protein | [A/G] | A | NS | 10.7251 | $2.6x10^{-5}$ | 0.0046 | 0.0237 |
| Prin1 | *sSPcDFE049E11411*-306 | pentatricopeptide-containing protein | [A/G] | A | NS | 5.7165 | 0.0035 | 0.0970 | 0.0125 |
| Prin2 | *60s RPL31a*-418 | 60s RPL31a | [A/G] | --- | Syn | 10.8028 | $2.5x10^{-5}$ | 0.0045 | 0.0335 |
| Prin2 | *60s RPL31a*-55 | 60s RPL31a | [A/G] | --- | NC | 9.2166 | 0.0001 | 0.0132 | 0.0285 |
| Prin2 | *CN637339.1*-337 | unknown hypothetical protein | [A/G] | A | NS | 6.0494 | 0.0025 | 0.0821 | 0.0182 |
| Prin2 | *Pm_CL234Contig1*-156 | rab GTPase | [A/T] | T | NC | 6.8320 | 0.0012 | 0.0573 | 0.0206 |
| Prin3 | *4CL1*-520 | 4-coumarate:CoA ligase 1 | [A/G] | --- | NS | 8.1685 | 0.0003 | 0.0258 | 0.0216 |
| Prin3 | *apx*-288 | ascorbate hydroxylase | [A/C] | --- | NC | 7.2288 | 0.0008 | 0.0478 | 0.0194 |
| Prin3 | *CN636471.1*-437 | phenylalanine ammonia-lyase | [C/G] | C | Syn | 5.8518 | 0.0030 | 0.0902 | 0.0158 |
| Prin3 | *CN637306.1*-381 | MYB-like transcription factor | [A/G] | A | Syn | 6.8751 | 0.0011 | 0.0569 | 0.0182 |
| Prin3 | *erd15*-635 | early response to dehydration protein | [A/C] | --- | NC | 5.9931 | 0.0026 | 0.0838 | 0.0159 |
| Prin3 | *LEA-EMB11*-263 | late embryogenesis abundant protein | [A/C] | --- | NC | 22.6840 | $2.4x10^{-6}$ | 0.0014 | 0.0310 |
| Prin3 | *Pm_CL2133Contig1*-144 | mitochondrial transcription termination factor | [C/G] | C | Syn | 5.8364 | 0.0031 | 0.0916 | 0.0155 |
| Prin3 | *Pm_CL2133Contig1*-305 | mitochondrial transcription termination factor | [A/T] | A | NS | 5.8065 | 0.0032 | 0.0923 | 0.0154 |
| Prin4 | *60s RPL31a*-55 | 60s RPL31a | [A/G] | --- | NC | 5.6490 | 0.0037 | 0.0998 | 0.0180 |

[a]Listed is the ancestral state as determined by comparison to a single sequence of bigcone Douglas-fir (*Pseudotsuga macrocarpa*). Dashes indicate that an outgroup sequence was unavailable.

[b]NC, noncoding; NS, nonsynonymous; Syn, synonymous.

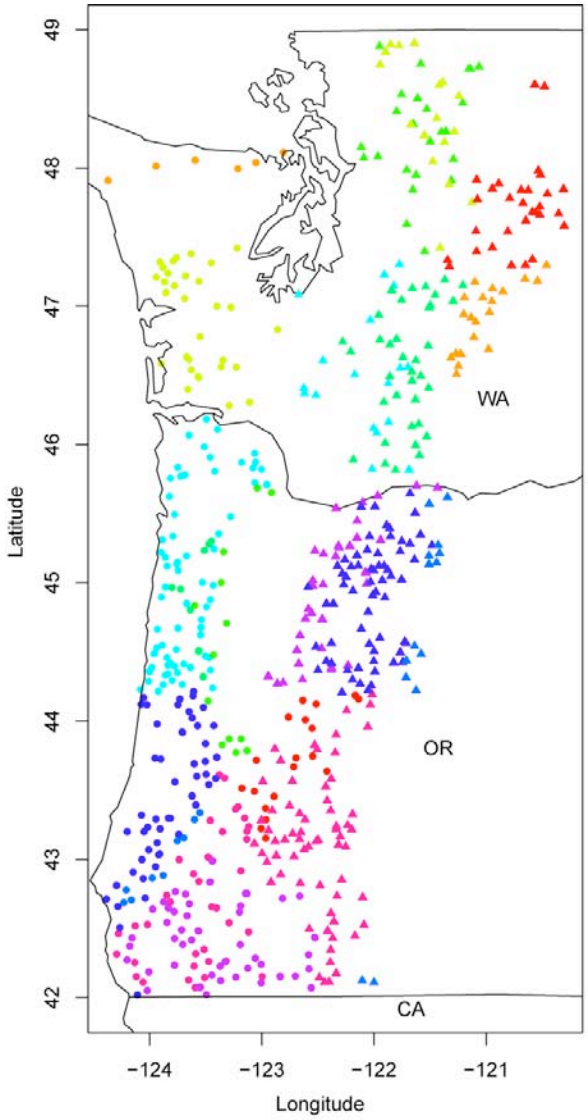


FIGURE S1.–An illustration of the 20 populations defined for the hierarchical analysis of molecular variance (AMOVA). Colors and point types (circles and triangles) designate different populations. Colors are recycled across point types, so that, for example, there are pink circles and pink triangles. The red and orange triangles located in northeastern Washington represent the 57 families located east of the Cascade crest.

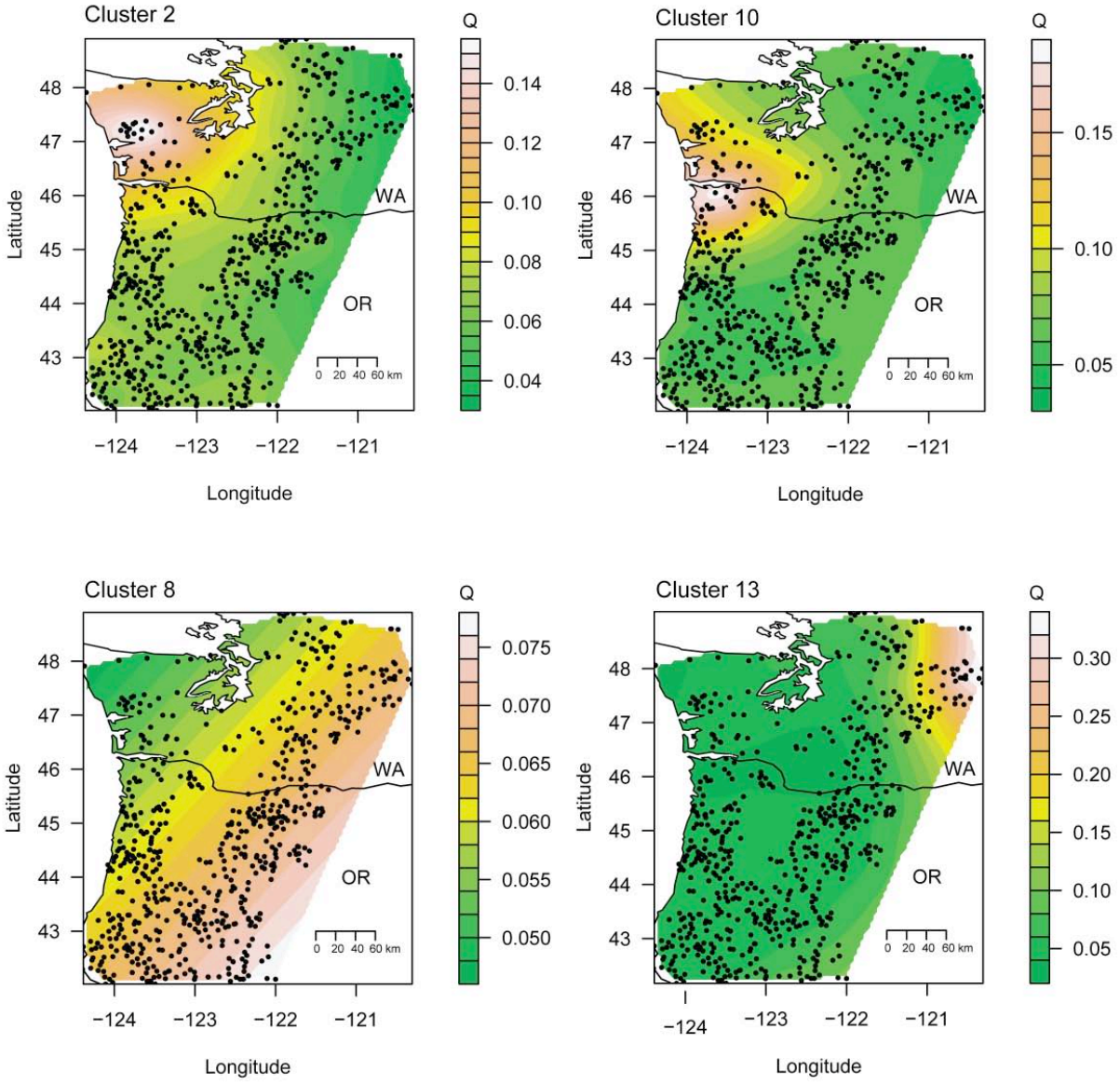


FIGURE S2.–An illustration of geographic trends in population structure for coastal Douglas-fir. For each of the 15 clusters, *Q*-values were smoothed with universal Kriging interpolation. Two patterns are apparent – a southwest to northeast trend (clusters 8 and 13) and a cluster centered on the coast of Washington (clusters 2 and 10). Points mark sampled mother trees ($n$ = 700).

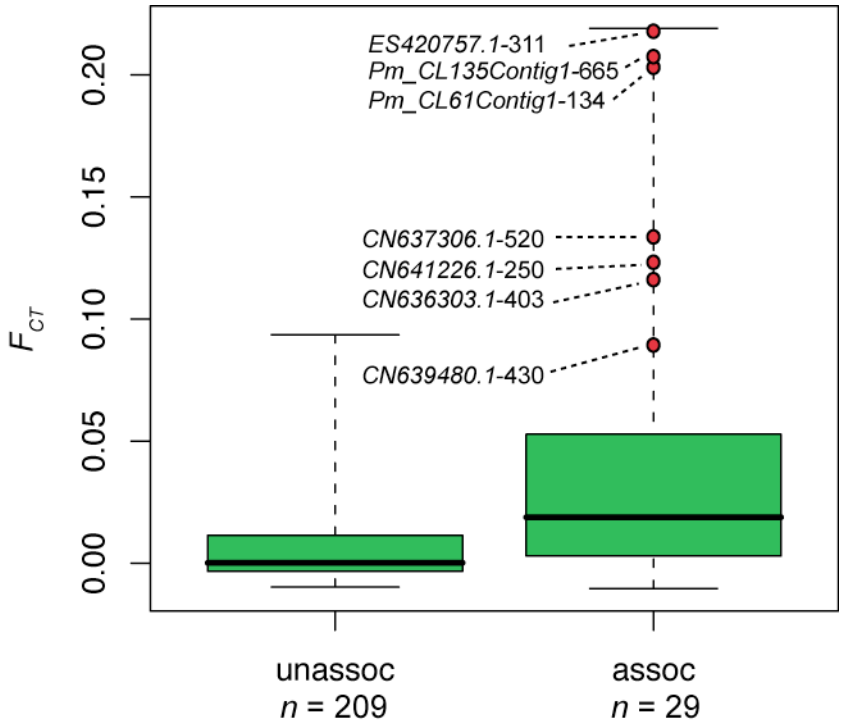


FIGURE S3.–Boxplots of the distribution of population structure estimates ($F_{CT}$) for markers associated with at least one trait when the 57 eastside families are included in the analysis (assoc) versus those that remain unassociated regardless of whether or not these families are included (unassoc). Dashed lines extend to the data extremes. The former class has a set of 15 markers removed due to overlap with the associations presented for the reduced data set. Thus, these 29 markers produce unique associations when the 57 families under consideration are included in the analysis. The latter class includes 25 allozyme markers from KRUTOVSKY *et al.* (2009). Points denote those markers with extreme levels of differentiation. They account for 40% of the increased number of associations.

## FILES S1–S5

Files S1 through S5 are available for download at http://www.genetics.org/cgi/content/full/genetics.109.102350/DC1. These text files contain the Primer sequences used for SNP discovery and Illumina genotyping.