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# Low genetic diversity, moderate local adaptation, and phylogeographic insights in *Cornus nuttallii* (Cornaceae)[1]

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- *Premise of the study:* Genetic knowledge is completely lacking for Pacific dogwood (*Cornus nuttallii*), a western North American temperate tree that is pollinated and dispersed by biological vectors. We investigated how history, geography, and climate have affected population genetic structure, local adaptation, and the phylogeography of this species.
- *Methods:* We examined patterns and levels of diversity in nuclear microsatellites (SSRs) and cpDNA haplotypes in populations from across the species range. We compared these results to population differentiation and genetic clines in phenotypic traits in a common garden.
- *Key results:* Genetic diversity was low for both nuclear SSRs and cpDNA. There was a lack of population structure ($F_{ST}$ = 0.090) in the coastal portion of the species range, with estimates of population genetic diversity in microsatellite markers decreasing with latitude from California to British Columbia. A disjunct interior population in Idaho 450 km from the coastal range had the lowest diversity but the highest divergence of all populations studied. Only a single nucleotide polymorphism was discovered after sequencing 5547 base pairs in seven noncoding regions of cpDNA. Both cpDNA haplotypes were widely distributed throughout the species range. Quantitative variation among populations was moderate ($0.11 \leq Q_{ST} \leq 0.63$), and weak but significant adaptive clines were found between quantitative traits and population climatic variables ($0.09 \leq R^2 \leq 0.34$).
- *Conclusions: Cornus nuttallii* likely faced a population bottleneck in a single southern refugium during the Last Glacial Maximum. Despite low genetic diversity, it is weakly to moderately locally adapted.

**Key words:** adaptive cline; chloroplast diversity; Cornaceae; *Cornus nuttallii*; microsatellite markers; Pacific dogwood; phenotypic variation; population structure; postglacial recolonization.

A complex natural history and geography have shaped patterns of population genetic structure in many western North American species (Brunsfeld et al., 2001; Shafer et al., 2010), while spatially heterogeneous selection pressures have left the phenotypic signatures of local adaptation in many temperate trees (Howe et al., 2003; Savolainen et al., 2007). The rangewide pattern of genetic and phenotypic diversity for a species can provide insight into the relative roles of evolutionary forces such as selection, gene flow, and genetic drift within a historical, geographic, and climatic context. Patterns of diversity also have implications for seed-transfer guidelines for species-specific management including reforestation and conservation. By combining population and quantitative genetics, we seek to explore the influences of history, geography, and climate on evolutionary forces that have created current genetic and phenotypic patterns across the range of *Cornus nuttallii,* a tree species for which genetic information is entirely lacking.

Pleistocene glaciation profoundly impacted the population genetic structure of plant and animal species in western North America (Shafer et al., 2010). Sympatric species within this region often show similar phylogeographic patterns, suggesting that many of these species shared glacial refugia and postglacial recolonization routes (Soltis et al., 1997; Brunsfeld et al., 2001). One commonly observed phylogeographic pattern is north–south partitioning of cpDNA haplotypes (Soltis et al., 1997; Brunsfeld et al., 2001). The most common division between these two geographic groups occurs in central to southern Oregon, at a boundary referred to as the "Soltis line" (Brunsfeld et al., 2007). North–south partitioning can be explained by postglacial recolonization either from multiple refugia (the "north–south" recolonization hypothesis), or from a single southern refugium with fixation of a distinct northern haplotype as recolonization progressed (the "leading edge" hypothesis) (Soltis et al., 1997). However, evidence is emerging that many northwestern North American species may have survived in multiple refugia, cryptic refugia, and subrefugia within larger refugial regions, suggesting that postglacial recolonization in many species has been more complex than these simple hypotheses would imply (Shafer et al., 2010). Additionally, the distributions of many coastal western North American species also include disjunct populations in the mesic interior forests between the Cascade and Rocky Mountains (Carstens et al., 2005). These populations may have originated through ancient vicariance (e.g., uprising of the Cascade Mountains during the late

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Pliocene) or by more recent dispersal from either a northern or southern source (Carstens et al., 2005).

Most tree species have high levels of genetic diversity due to large effective population sizes, high reproductive capabilities and long life spans (Petit and Hampe, 2006), and most populations of temperate and boreal tree species are moderately to strongly adapted to local environments (Howe et al., 2003; Savolainen et al., 2007). Adaptive clines in phenology, morphology, growth, and stress resistance are commonly observed in forest trees along geographic and climatic gradients (Howe et al., 2003; Savolainen et al., 2007). Despite recurring patterns of local adaptation, not all temperate tree species are strongly locally adapted (e.g., *Pinus monticola* Douglas ex D. Don: Rehfeldt et al., 1984; Meagher and Hunt, 1998). Capacity for local adaptation may be limited by several factors, including high rates of among-population gene flow (Brown and Pavlovic, 1992) and reduced genetic diversity due to historical population bottlenecks (England et al., 2003). However, phenotypic variation in quantitative traits is typically controlled by many genes with small effects (González-Martínez et al., 2006), which could increase the potential for rapid local adaptation to new conditions, even in situations of reduced overall genetic diversity (Lande, 1995; Aitken et al., 2008). Some species may also tolerate environmental heterogeneity through phenotypic plasticity.

Tree species that employ biological vectors for both pollination and seed dispersal have been underrepresented in forest genetic studies in western North America. The commercial value of conifers in this region, which typically use wind for both pollen and seed dispersal, has made these trees the primary focus of population genetic studies. Studies of angiosperm species utilizing animal pollination and seed-dispersal mechanisms may broaden our understanding of the full range of tree evolutionary dynamics and resultant geographic patterns of diversity.

Pacific dogwood (*Cornus nuttallii* Audubon ex Torr. & A. Gray) is a tree species endemic to western North America. Its distribution extends from the lowlands of southwestern British Columbia to the mountains of southern California (Fig. 1). It is found disjunctly in northern Idaho and in the transverse ranges of southern California. Unlike many sympatric tree species, *C. nuttallii* is pollinated by insects, and its seeds are ingested and dispersed by birds and mammals (Klinka et al., 2000). Genetic literature is entirely absent for this species.

We present the results of chloroplast-sequence and nuclear-microsatellite analyses and compare them to patterns of local adaptation and population differentiation in phenotypic traits observed in a common garden experiment. These data are used to address the following questions: (1) Does *C. nuttallii* exhibit high nuclear and chloroplast genetic diversity like many tree species? (2) Is *C. nuttallii* structured into northern and southern genetic groups, similar to many other western North American plant species? (3) Is there evidence from phenotypic clines that *C. nuttallii* is strongly locally adapted to climate, like most temperate and boreal tree species, and if so, are phenotypic clines consistent with those observed in other trees? (4) Where were glacial refugia located, and what was the likely postglacial recolonization route of *C. nuttallii*? The results of this study will inform efforts to conserve genetic diversity in this species and to select seed sources for restoration and reforestation, and help predict its capacity for response to climate change.

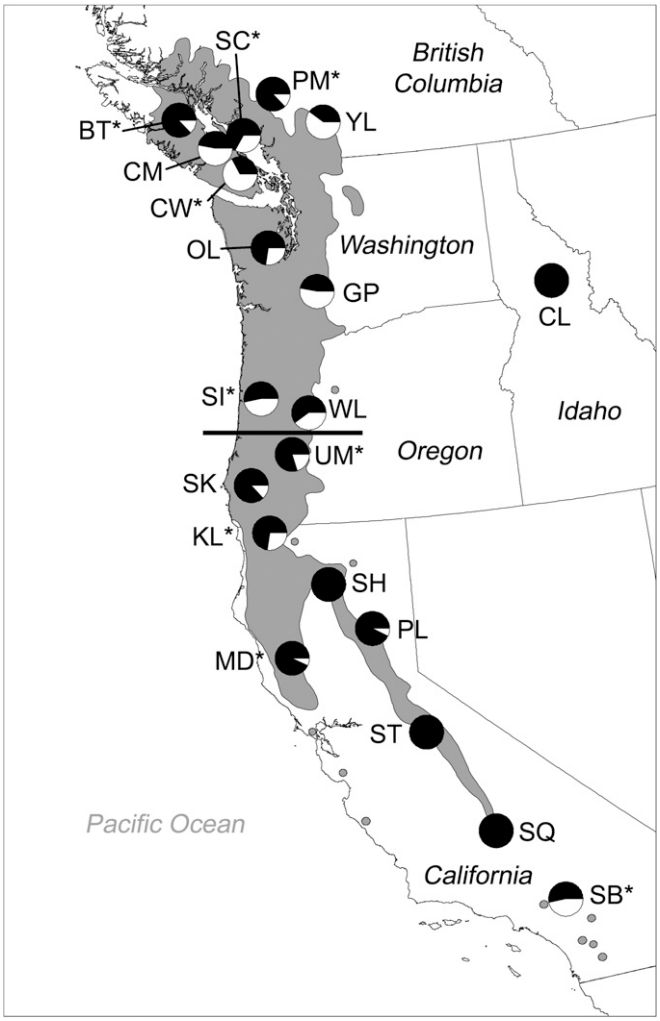


Fig. 1. Geographic distribution and frequencies of chloroplast haplotypes (black, haplotype 1; white, haplotype 2) in each sampled population (two-letter ID) throughout the native range of *Cornus nuttallii* (gray shading). The solid black line corresponds roughly to the Soltis line (Brunsfeld et al., 2007). Many phylogeographic studies have found distinct frequencies of cpDNA haplotypes on either side of this hypothetical line (Soltis et al., 1997). Populations marked with an asterisk (*) were also represented in the common garden and were included in analyses of quantitative traits.

## MATERIALS AND METHODS

***Sampling methods—DNA sequence analysis***—Fresh foliage was sampled from 595 individuals from 20 native populations during April and May 2006 (Table 1, Fig. 1). These populations span the entire geographic range of the species. Two disjunct populations were sampled: one at the southern limit of the species range in the San Bernardino Mountains in southern California (SB) and another at the eastern species limit in the Clearwater National Forest in northern Idaho (CL). Populations were spaced a minimum of 75 km apart, with most separated by much larger distances. Sampled populations in United States Forest Service National Forests were located using the Pacific Northwest Forest Inventory and Analysis database (Waddell and Hiserote, 2005) and local knowledge. Because *C. nuttallii* occurs at relatively low densities, the sampling strategy employed was opportunistic. Twenty-six to 31 individual trees, spaced a minimum of 30 m apart, were sampled from each population. Leaves were frozen in liquid nitrogen in the field and stored at –80°C. All 595 sampled individuals were used in the microsatellite study, while 15 individuals per population were randomly selected for chloroplast sequencing.

TABLE 1. *Cornus nuttallii* provenance locations, leaf tissue sample sizes, and estimated geographic and climatic variables.

| Provenance | ID | *N* | Lat. (°N) | Long. (°W) | Elev. (m a.s.l.) | MAT (°C) | MWMT (°C) | MCMT (°C) | MAP (mm) | MSP (mm) | FFP (days) | GDD ≥ 5°C |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| San Bernardino NF, CA | SB [a] | 30 | 34.24 | 117.20 | 1684 | 11.3 | 20.8 | 3.8 | 984 | 75 | 176 | 2484 |
| Sequoia NF, CA | SQ [a, b] | 28 | 35.72 | 118.54 | 1722 | 11.5 | 21.2 | 4.7 | 638 | 50 | 174 | 2519 |
| Stanislaus NF, CA | ST | 30 | 37.81 | 119.92 | 1153 | 12.9 | 22.4 | 5.4 | 1004 | 77 | 207 | 2967 |
| Plumas NF, CA | PL | 30 | 39.91 | 121.01 | 1245 | 10.3 | 19.9 | 1.8 | 1310 | 105 | 133 | 2263 |
| Mendocino NF, CA | MD [a] | 30 | 39.43 | 122.99 | 725 | 13.2 | 21.9 | 5.8 | 1239 | 71 | 191 | 3104 |
| Shasta-Trinity NF, CA | SH | 30 | 40.84 | 122.01 | 418 | 15.8 | 24.8 | 6.6 | 1436 | 137 | 265 | 4021 |
| Klamath NF, CA | KL [a] | 30 | 41.88 | 123.41 | 543 | 11.7 | 20.5 | 4.4 | 1481 | 123 | 185 | 2585 |
| Siskiyou NF, OR | SK | 26 | 42.80 | 123.83 | 818 | 10.9 | 18.6 | 5.6 | 3134 | 264 | 230 | 2265 |
| Umpqua NF, OR | UM [a] | 30 | 43.38 | 122.75 | 425 | 11.7 | 19.7 | 5.0 | 1444 | 225 | 217 | 2573 |
| Willamette NF, OR | WL | 30 | 44.16 | 122.26 | 366 | 11.3 | 19.5 | 3.8 | 1406 | 239 | 183 | 2469 |
| Siuslaw NF, OR | SI [a] | 30 | 44.46 | 123.51 | 408 | 10.5 | 18.4 | 4.1 | 2003 | 231 | 215 | 2206 |
| Clearwater NF, ID | CL [a, b] | 31 | 46.23 | 115.46 | 523 | 9.3 | 20.6 | −1.7 | 1051 | 302 | 151 | 2245 |
| Gifford-Pinchot NF, WA | GP | 30 | 46.48 | 121.87 | 640 | 8.9 | 17.4 | 1.2 | 1869 | 318 | 164 | 1833 |
| Olympic NF, WA | OL | 30 | 47.36 | 123.16 | 60 | 10.7 | 18.0 | 4.3 | 1760 | 239 | 215 | 2237 |
| Lake Cowichan, BC | CW [a] | 30 | 48.79 | 123.89 | 231 | 9.1 | 16.9 | 2.2 | 1515 | 213 | 191 | 1786 |
| Cameron Lake, BC | CM | 30 | 49.29 | 124.58 | 215 | 7.9 | 15.8 | 1.2 | 1746 | 259 | 157 | 1536 |
| Sechelt, BC | SC [a] | 30 | 49.53 | 123.75 | 89 | 9.2 | 17.1 | 2.5 | 1831 | 388 | 221 | 1815 |
| Yale, BC | YL | 30 | 49.70 | 121.40 | 149 | 8.1 | 18.7 | −2.5 | 1368 | 261 | 157 | 1959 |
| Buttle Lake, BC | BT [a] | 30 | 49.83 | 125.62 | 242 | 7.9 | 16.6 | 0.7 | 1665 | 274 | 171 | 1621 |
| Pemberton, BC | PM [a] | 30 | 50.29 | 122.84 | 402 | 6.5 | 16.9 | −3.7 | 1549 | 304 | 132 | 1597 |

*Notes:* BC, British Columbia; CA, California; elev., elevation above sea level; FFP, frost-free period; GDD, growing degree days; ID, Idaho; lat., latitude; long., longitude; MAT, mean annual temperature; MWMT, mean warmest-month temperature; MCMT, mean coldest-month temperature; MAP, mean annual precipitation; MSP, mean summer precipitation; NF, United States Forest Service National Forest; OR, Oregon; WA, Washington.

[a] Sampled for both foliage for genetic marker analysis and seeds for common garden.

[b] Excluded from quantitative trait analyses due to insufficient common-garden sample size.

*Phenotypic trait analysis*—Seeds were collected from 164 individuals from 11 of the 20 populations in the fall of 2006 (Table 1). Single-seeded fruits were picked from multiple inflorescences from a minimum of 10 trees per population, spaced at least 30 m apart. While the same locations were sampled for both leaf tissue and seeds, individual trees were not identified for repeated sampling on both dates.

***DNA extraction and microsatellite marker development and genotyping***—Total genomic DNA from leaf samples was isolated using a modified CTAB method (Doyle and Doyle, 1987). As no microsatellite markers had been developed specifically for *C. nuttallii*, eight primers from *C. florida*, *C. nuttallii*'s closest relative, were used (Xiang et al., 1996, 2006; Cabe and Liles, 2002). Sequence data for eight additional microsatellite loci characterized in *C. florida* were obtained through the GenBank database (AF387361, AF387358, AF356102, AF356094, AF356093, AF356091, AF387359, and AF356096), and primers were designed. In a screen of four individuals per population from all 20 populations, only nine of the 16 *C. florida* loci produced scorable bands in *C. nuttallii*, of which only five were found to be polymorphic (*Cn-G4*, *Cn-J7*, *Cn-N4*, *Cn-N5*, and *Cn-N10*). To confirm short sequence repeats in *C. nuttallii*, we sequenced PCR products from two homozygous individuals using SequiTherm EXCEL II Long-Read DNA Sequencing kits-LC (Epicentre Bioechnologies, Madison, Wisconsin, USA) on a LiCor 4200 automated sequencer (LiCor, Lincoln, Nebraska, USA).

Polymerase chain reactions (PCRs) were performed using an MJ Research PTC-100 thermal cycler (MJ Research, Waltham, Massachusetts, USA). Each reaction had a total volume of 10 μL and contained 40 ng of total genomic DNA, 1.0 μL of 2.0 mM dNTP, 1.0 μL 10× *Paq*5000 Reaction Buffer (Stratagene, La Jolla, California, USA), 1 U *Paq*5000 DNA polymerase (Stratagene), 0.5–0.8 pmol of M13 IR Label Primer (LiCor) and tailed primers (0.5–1.0 pmol each). Samples were amplified using the following PCR program: 2 min at 94°C, followed by 30 cycles of 30 s at 94°C, 45 s at the optimal annealing temperature (Table 2A), 30 s at 72°C, and an extension cycle of 5 min at 72°C. Amplification products were electrophoresed on 5% Long Ranger polyacrylamide gels (Lonza, Basel, Switzerland) using a LiCor 4200 automated sequencer. Bands were scored using Gene ImagIR RFLP with 100 bp ladders (LiCor).

Standard measures of genetic diversity, including average number of alleles per locus (allelic richness, $A_R$), observed heterozygosity ($H_O$), unbiased expected heterozygosity ($H_E$), and inbreeding coefficient of individuals relative the population ($F_{IS}$) and total ($F_{IT}$) were estimated for each population and overall using GenAlEx software (Peakall and Smouse, 2006). Estimates of $F_{ST}$ (Weir and Cockerham, 1984) and the microsatellite-specific $R_{ST}$ that utilizes allele length information (Slatkin, 1995) were also calculated over all populations by the AMOVA method (Excoffier et al., 1992). $F_{ST}$ was calculated for each individual population as averages of pairwise estimates between populations. Mantel tests (Mantel, 1967) were used to test for isolation by distance in the coastal portion of the species range (excluding the disjunct Idaho population), by estimating the correlation between linearized estimates of genetic distance, expressed as $F_{ST}/(1 - F_{ST})$ or $R_{ST}/(1 - R_{ST})$, and geographic distance (Rousset 1997). Linear regressions were performed on alleles per locus, observed heterozygosity, and expected heterozygosity vs. latitude for coastal populations only. All response variables were square transformed to achieve a normal distribution of residuals.

***Chloroplast sequencing and analysis***—Universal primers were employed to amplify seven different regions of the chloroplast genome for sequencing (Table 2B). These regions had previously been mapped to several different physical locations in the *Nicotiana* chloroplast genome (Wakasugi et al., 1998) and had demonstrated intraspecific variation in other asterids (Shaw et al., 2005). During preliminary sequencing, 5547 base pairs (bp) of the *C. nuttallii* chloroplast genome were sequenced in 10 individuals (two individuals per population from five geographically distant populations). Although no estimates of the size of this species' chloroplast genome have been published, based on the size range for angiosperms in general it is likely that between 7.6 and 10.8% of the noncoding elements were sequenced. Chloroplasts were assumed to be maternally inherited in *C. nuttallii*, as in most angiosperms (Corriveau and Coleman, 1988).

Preliminary sequences were obtained using SequiTherm EXCEL II Long-Read DNA Sequencing kits-LC (Epicentre Biotechnologies), using PCR programs outlined by Shaw et al. (2005), on a LiCor 4200 automated sequencer. Nested primers were designed with the online version of the program Primer 3 (Rozen and Skaletsky, 2000), using data from preliminary sequences, to amplify suspected variable regions. These nested regions were amplified and sequenced in 100 randomly selected trees from 10 populations (10 individuals per population), covering the entire range of *C. nuttallii*. Only one region (*rpl16*) showed nucleotide sequence variation in the form of a single base pair substitution and was then sequenced in a total of 300 individuals (15 individuals per population) for all 20 sampled populations. All sequencing was performed at the Genome Sciences Centre in Vancouver, British Columbia, Canada.

TABLE 2. Primers, PCR conditions, and details of amplified polymorphic sites in *Cornus nuttallii* for (A) microsatellite loci and (B) chloroplast loci.

| (A) Locus | Primer sequence (5′–3′) | $T_a$ (°C) [a] | M13 (μL) | [Mg] (mM) | Repeat motif | Fragment size range (bp) | Alleles (*n*) | GenBank accession |
|---|---|---|---|---|---|---|---|---|
| *Cn-J7* [c] | AACACTGCCCCATTGTTAGAG<br>GAGGTGTCTCTCTCGTGGTTC [b] | 55 | 0.5 | 1.5 | $(TC)_{14}(AC)_{10}$ | 126–132 | 4 | DQ223104 |
| *Cn-G4* [d] | TCATGCCCCGCTAAACCGAC [b]<br>CATCACTGTACTCAGGCC | 49 | 20 | 0.5 | $(TC)_{11}$ | 134–140 | 3 | DQ223112 |
| *Cn-N5* [c] | GCAAGGATCGAACTTAGGG<br>TGAATTATGTATCGAATGTCTGC [b] | 59 | 0.5 | 2.5 | $(AT)_{11}$ | 122–128 | 4 | DQ223107 |
| *Cn-N10* [c] | TGATTGAATAACCTTTTGATGC [b]<br>GGTAGCTTCAAATGTCAACG | 55 | 0.5 | 2.5 | $(TA)_{25}(TG)_{12}$ | 184–210 | 14 | DQ223108 |
| *Cn-N4* [d] | TGGCCTTTGGAGAGGGAATGCA<br>CATTCCCAGATGTTTGGTCTATC [b] | 62 | 0.5 | 2.5 | $(GA)_{11}$ | 251–253 | 2 | DQ223110 |

| (B) Region of chloroplast | Primer name | $T_a$ (°C) [a] | Reference | GenBank accession |
|---|---|---|---|---|
| *trnH*$^{GUG}$-*psbA* | trnH$^{GUG}$, psbA | 53 | Hamilton, 1999 | FJ541997 |
| *rps16* | rps16F, rps16R | 53 | Shaw et al., 2005 | FJ541998 |
| 5′*rps12*-*rpl*20 | 5′rps12, rpl20 | 53 | Hamilton, 1999 | FJ541999 |
| *psbB*-*psbH* | psbB, psbH | 58 | Shaw et al., 2005 | FJ542000 |
| *rpl16* | rpL16F71, rpL16R1516 | 50 | Shaw et al., 2005 | FJ542003-FJ542004 |
| 5′*trnL*$^{UAA}$-*trnT*$^{UGU}$ (within 5′*trnL*$^{UAA}$-*trnS*$^{UGA}$) | 5′trnLR (Tab B), trnTF (Tab A) | 55 | Shaw et al., 2005 | FJ542001 |
| *rps*4R2-*trnT*$^{UGU}$(within 5′*trnL*$^{UAA}$-*trnS*$^{UGA}$) | rps4R2, trnTR | 55 | Shaw et al., 2005 | FJ542002 |

[a] Annealing temperature used in PCR.
[b] Primer on which a forward or reverse tail sequence was used.
[c] From Cabe and Liles (2002); originally discovered in *C. florida*.
[d] Designed from GenBank sequences.

Trace files were viewed and quality judged using the program FinchTV (Geospiza, Seattle, Washington, USA). Sequences were aligned using Molecular Evolutionary Genetic Analysis (MEGA), version 4.0 (Tamura et al., 2007). Overall haplotype diversity (*h*) and unbiased Nei's pairwise population genetic distances *(D)* were calculated using Genetic Analysis in Microsoft Excel version 6.1 (GenAlEx) (Peakall and Smouse, 2006).

***Common garden design***—A common garden was established in Vancouver, British Columbia, Canada, using seeds collected from open-pollinated progeny of sampled parent trees (open-pollinated families) within populations from across the entire species range (Table 1). All fruits were soaked in concentrated sulphuric acid for 2 h, immersed in running water for 48 h, and transferred to moistened peat at room temperature for a 30-d warm stratification followed by a 30-d cold stratification at 4°C. Cold stratification was terminated sooner than planned because some seeds had begun to germinate. Seeds were sown in containers in a greenhouse in January 2007, then transplanted outdoors into raised beds 75 cm deep in June of the same year. The outdoor common garden was planted in a randomized blocked design, with seedlings from each open-pollinated family within population distributed evenly among the 12 blocks and randomly within each block. A total of 991 seedlings from 130 open-pollinated families within 11 populations were planted in the common garden. However, due to seed predation by rodents in the greenhouse and additional mortality as the study progressed, the experiment was unbalanced, and sample sizes for quantitative trait measurements were often substantially smaller. Populations represented by fewer than three open-pollinated families and open-pollinated families represented by fewer than three individuals were excluded from all statistical analyses.

***Quantitative trait phenotyping***—Individuals were phenotyped for 10 traits related to growth, phenology, and cold hardiness. Selected traits were a priori candidates for involvement in local adaptation, due to their moderate to high population differentiation ($Q_{ST}$) observed in other tree species, reflecting a tradeoff between competitive ability (growth) and avoidance of cold injury (Howe et al., 2003; Savolainen et al., 2007).

Growth and phenology traits assessed included first- and second-year height, diameter, and bud-flush and bud-set stage ($N$ = 536 trees; 73 open-pollinated families within nine populations). First- and second-year heights were measured after the 2007 and 2008 growing seasons, respectively. The height of each tree was measured on eight dates throughout the second growing season in 2008. Diameter was measured after the 2009 growing season 15 cm above the root collar. Bud-flush stage of the terminal bud was assessed in late March 2008, at the beginning of the second growing season. A scale of 1 through 6 was used, with chronological stages represented as follows: 1, shoot extended and damaged by frost in situ; 2, shoot extending but no frost damage; 3, individual leaves unfurling from bud; 4, green tip longer in length than bud scale but no leaves unfurled; 5, green tip visible but shorter in length than bud scale; and 6, no signs of bud flush. In late September at the end of the same growing season, bud-set stage of the terminal bud was assessed as follows: 1, fully formed, hardened, pointy bud; 2, pointy bud forming but still soft with small amount of green tissue visible; 3, small bud just beginning to form; and 4, no signs of bud set.

Estimates were also obtained for three additional phenological traits derived from periodic height growth measurements ($N$ = 622 trees; 71 open-pollinated families within nine populations). A sigmoidal growth curve was fit to the incremental 2008 height growth measurements for each individual tree (Rehfeldt and Wykoff, 1981), using PROC MODEL in SAS version 9.2 (SAS Institute, Cary, North Carolina, USA). The traits then estimated from the sigmoidal growth curves were Julian dates of the year by which 5% and 95% of a tree's seasonal height growth had occurred (dates of 5% and 95% growth, respectively), and the duration of time between these two dates (duration of 5–95% growth).

Fall cold hardiness was assessed for a subset of 359 trees from 46 open-pollinated families within nine populations located in the common garden. Artificial freeze tests were used to estimate the index of injury ($I_T$) at temperature $T$, and the estimated low temperature at which 50% tissue lethality occurs ($LT_{50}$), using a modified version of the procedure described by Hannerz et al. (1999). Branch cross-sectional samples ca. 1 mm thick were sampled in November 2008. Samples were frozen to –11°C, –18°C and –25°C, and electrolyte leakage measurements were used to calculate $I_T$ (Flint et al., 1967). Because genetic variation was greatest at –25°C, only $I_{-25}$ was used in subsequent analyses. In addition to $I_{-25}$, the 50% lethal temperature ($LT_{50}$) was also estimated by linear interpolation between test temperatures.

***Analyses of quantitative traits***—All statistical analyses were conducted using procedures in SAS version 9.2. Simple linear regressions were performed, comparing open-pollinated family means of quantitative phenotypic traits to geographic and climatic variables associated with parent trees using PROC REG. Due to the large number of regressions, a sequential Bonferroni correction for multiple comparisons (Rice, 1989) was performed at the level of each quantitative trait, with a trait-wise significance level of $\alpha = 0.05$. Geographic and climatic variables included in regressions are summarized in Table 1. Climatic variables for each population's provenance were estimated

using the program ClimateWNA version 4.60, an extension of ClimateBC (Wang et al., 2006).

Analyses of variance were also conducted for each quantitative trait according to the following model:

$$y_{ijkl} = \mu + b_i + p_j + b_i p_j + f(p)_{jk} + e_{ijkl},$$

where $y_{ijkl}$ is the observed quantitative trait value for individual $l$ in family $k$ within population $j$ in block $i$, $\mu$ is the overall quantitative trait mean, $b_i$ is the effect of block $i$, $p_j$ is the effect of population $j$, $b_i p_j$ is the effect of interaction between block $i$ and population $j$, $f(p)_{jk}$ is the effect of open-pollinated family $k$ nested within population $j$, and $e_{ijkl}$ is the residual error.

Population least square means were calculated using PROC GLM, and variance components were estimated using PROC VARCOMP with restricted maximum likelihood (METHOD = REML) using type III sums of squares. Variance components were used to calculate the among-population genetic differentiation in quantitative traits, $Q_{ST}$ (Spitze, 1993). We assumed that progeny within open-pollinated families were one-third identical by descent on average and, therefore, estimated additive genetic variance ($V_A$) as three times the family variance ($V_F$) for calculating $h^2$ and $Q_{ST}$ (Bower and Aitken, 2006). Open-pollinated progeny that are true half sibs experiencing no inbreeding and no correlated paternity would be one-quarter identical by descent, yet these assumptions are unlikely to hold for open-pollinated families of forest trees (Squillace, 1974). One-third identity-by-descent is a reasonable approximation assuming moderate inbreeding and some level of correlated paternity (Bower and Aitken, 2006).

## RESULTS

***Microsatellite diversity and divergence***—A total of 27 alleles were detected at the five microsatellite loci across all 20 populations (Appendix S1; see Supplemental Data with the online version of this article). Eight of these alleles were shared among all 20 populations, and no private alleles were observed. Genetic diversity parameters calculated over all populations and loci included allelic richness ($A_R = 3.7 \pm 0.3$), observed heterozygosity ($H_O = 0.281 \pm 0.018$), and unbiased expected heterozygosity ($H_E = 0.468 \pm 0.022$) (Table 3). Inbreeding coefficients of individuals relative to population ($F_{IS}$) and to the total ($F_{IT}$) were 0.393 (± 0.027), and 0.456 (± 0.118), respectively (Table 3). With the exclusion of the disjunct Idaho population (CL), there was a significant trend of diversity decreasing from south to north for $A_R$ ($R^2 = 0.21$, $P < 0.05$), $H_E$ ($R^2 = 0.46$, $P < 0.01$), and $H_O$ ($R^2 = 0.41$, $P < 0.01$) (Fig. 2).

Population differentiation estimates were 0.111 for $F_{ST}$ and 0.155 for $R_{ST}$. When the disjunct Idaho population (CL) was excluded from analyses, genetic distances among the remaining populations were much smaller, with $F_{ST} = 0.090$ and $R_{ST} = 0.063$. No significant isolation by distance was found between linearized differentiation statistics $F_{ST}/(1 - F_{ST})$ or $R_{ST}/(1 - R_{ST})$ and geographic distance when all populations were analyzed (Mantel test; $F_{ST}$: $R = 0.187$, $P = 0.11$; $R_{ST}$: $R = -0.031$, $P = 0.43$). When the CL population was excluded, significant isolation by distance was found between $F_{ST}/(1 - F_{ST})$ and geographic distance (Mantel test, $R = 0.596$, $P = 0.01$) but not $R_{ST}/(1 - R_{ST})$ and geographic distance (Mantel test, $R = 0.142$, $P = 0.14$). Population differentiation estimates for only those populations represented in the common garden for comparisons with quantitative-trait results were 0.090 and 0.102 for $F_{ST}$ and $R_{ST}$, respectively.

***Chloroplast diversity and divergence***—Even though 3–5% of the noncoding region of the chloroplast genome was sequenced in 100 individuals, only two haplotypes were identified. The haplotypes were distinguished by a single base pair (A or G) mutation 881 base pairs from the 5′ end of the sequenced *rpl16* region. Haplotype 1 occurred at a frequency of 0.735 across all individuals sequenced. Both haplotypes were present in all but four of the 20 populations (Fig. 1). Three of the California populations (SQ, ST, and SH) and the Idaho population (CL) were fixed for haplotype 1. Total unbiased haplotype diversity ($h_T$) for chloroplasts was 0.316 (± 0.046). Overall population genetic structure (Nei's unbiased genetic distance, $D$) averaged 0.153. When populations were separated into northern and southern groups to test for partitioning along the Soltis line (Brunsfeld et al., 2007), with the Idaho population included

TABLE 3. Estimates of within-population genetic diversity parameters (±SE) for 20 natural populations of *Cornus nuttallii*.

| Population | $A_R$ | $H_O$ | $H_E$ | $F_{IS}$ | $F_{IT}$ | $F_{ST}$ |
|---|---|---|---|---|---|---|
| SB | 4.2 ± 1.2 | 0.327 ± 0.078 | 0.515 ± 0.084 | 0.370 ± 0.114 | 0.447 ± 0.088 | 0.123 ± 0.065 |
| SQ | 3.4 ± 0.9 | 0.371 ± 0.060 | 0.527 ± 0.023 | 0.284 ± 0.113 | 0.375 ± 0.066 | 0.127 ± 0.068 |
| ST | 5.0 ± 1.8 | 0.293 ± 0.095 | 0.500 ± 0.113 | 0.445 ± 0.134 | 0.542 ± 0.139 | 0.175 ± 0.070 |
| PL | 4.4 ± 1.5 | 0.379 ± 0.071 | 0.579 ± 0.079 | 0.344 ± 0.040 | 0.394 ± 0.034 | 0.076 ± 0.057 |
| MD | 3.6 ± 0.9 | 0.307 ± 0.046 | 0.544 ± 0.077 | 0.426 ± 0.030 | 0.486 ± 0.040 | 0.104 ± 0.058 |
| SH | 4.2 ± 1.5 | 0.293 ± 0.095 | 0.554 ± 0.104 | 0.509 ± 0.072 | 0.547 ± 0.090 | 0.077 ± 0.068 |
| KL | 4.4 ± 1.5 | 0.300 ± 0.070 | 0.562 ± 0.085 | 0.478 ± 0.066 | 0.507 ± 0.072 | 0.056 ± 0.059 |
| SK | 4.0 ± 1.3 | 0.262 ± 0.072 | 0.419 ± 0.115 | 0.326 ± 0.123 | 0.391 ± 0.079 | 0.096 ± 0.077 |
| UM | 4.0 ± 1.3 | 0.307 ± 0.067 | 0.495 ± 0.106 | 0.358 ± 0.110 | 0.395 ± 0.072 | 0.058 ± 0.060 |
| WL | 3.6 ± 1.9 | 0.287 ± 0.109 | 0.432 ± 0.140 | 0.348 ± 0.112 | 0.394 ± 0.074 | 0.071 ± 0.073 |
| SI | 3.4 ± 1.4 | 0.313 ± 0.094 | 0.492 ± 0.105 | 0.384 ± 0.084 | 0.430 ± 0.063 | 0.074 ± 0.049 |
| CL | 2.2 ± 0.2 | 0.103 ± 0.059 | 0.245 ± 0.051 | 0.664 ± 0.186 | 0.764 ± 0.429 | 0.297 ± 0.067 |
| GP | 2.8 ± 1.1 | 0.267 ± 0.104 | 0.445 ± 0.131 | 0.391 ± 0.148 | 0.445 ± 0.115 | 0.088 ± 0.079 |
| OL | 3.8 ± 1.3 | 0.320 ± 0.087 | 0.427 ± 0.114 | 0.209 ± 0.110 | 0.271 ± 0.044 | 0.078 ± 0.076 |
| CW | 3.4 ± 0.9 | 0.247 ± 0.109 | 0.495 ± 0.085 | 0.500 ± 0.143 | 0.565 ± 0.169 | 0.129 ± 0.076 |
| CM | 4.2 ± 1.7 | 0.340 ± 0.092 | 0.484 ± 0.112 | 0.258 ± 0.111 | 0.308 ± 0.049 | 0.067 ± 0.050 |
| SC | 2.8 ± 0.6 | 0.240 ± 0.085 | 0.423 ± 0.092 | 0.331 ± 0.199 | 0.419 ± 0.131 | 0.131 ± 0.084 |
| YL | 3.4 ± 1.7 | 0.253 ± 0.084 | 0.397 ± 0.124 | 0.306 ± 0.088 | 0.401 ± 0.069 | 0.137 ± 0.099 |
| BT | 4.0 ± 1.5 | 0.180 ± 0.090 | 0.459 ± 0.126 | 0.641 ± 0.082 | 0.669 ± 0.166 | 0.079 ± 0.088 |
| PM | 3.6 ± 1.2 | 0.233 ± 0.074 | 0.375 ± 0.120 | 0.285 ± 0.168 | 0.363 ± 0.093 | 0.109 ± 0.093 |
| Overall | 3.7 ± 0.3 | 0.281 ± 0.018 | 0.468 ± 0.022 | 0.393 ± 0.027 | 0.456 ± 0.118 | 0.111 ± 0.054 |

*Notes:* See Table 1 for full names and details of populations. *A*R, allelic richness; $H_O$, observed heterozygosity; $H_E$, unbiased expected heterozygosity; $F_{IS}$, inbreeding coefficient of individuals relative to the population; $F_{IT}$, inbreeding coefficient of individuals relative to the total; $F_{ST}$, average pairwise differentiation between population and all other populations.

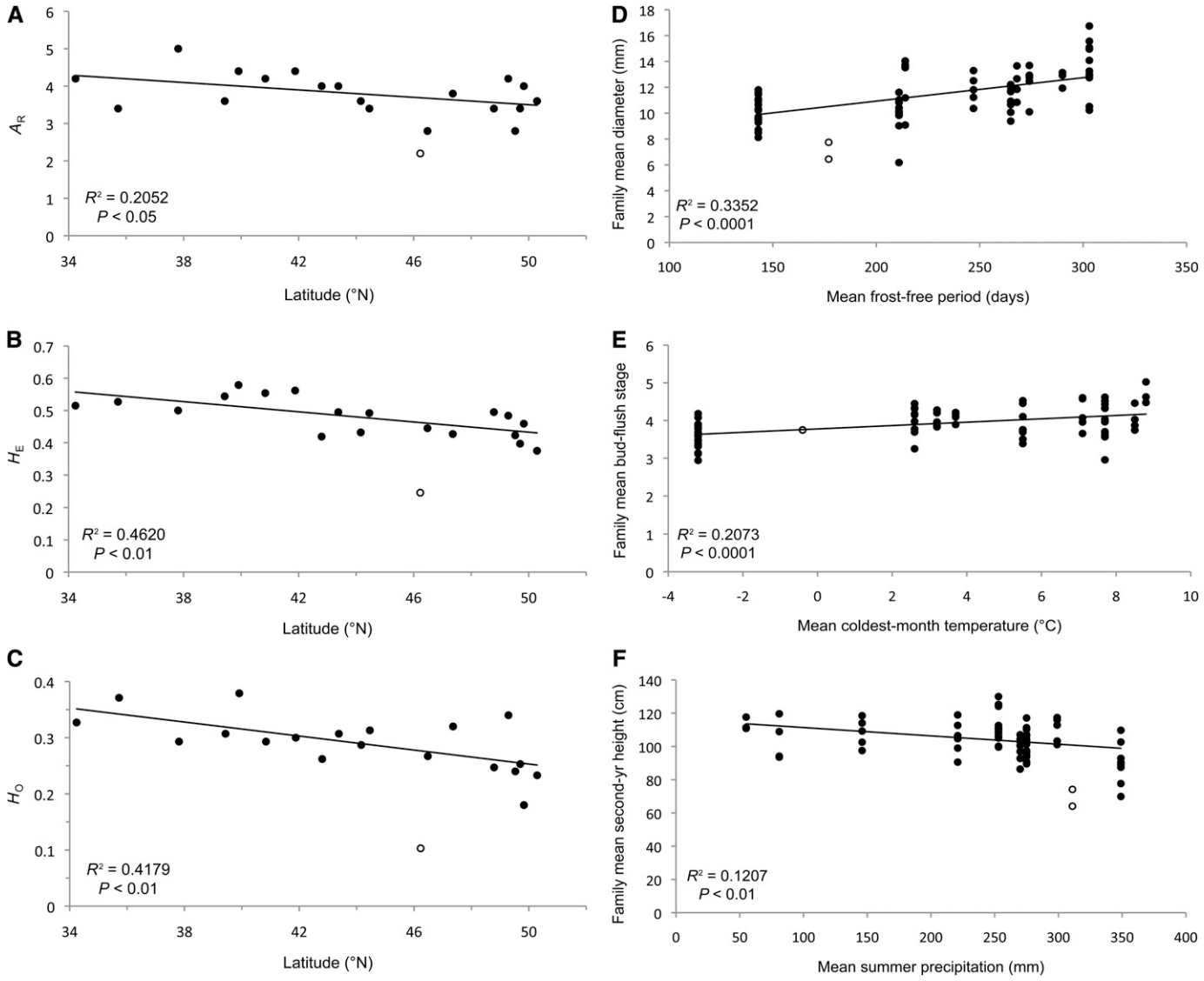


Fig. 2. Clines in molecular diversity and quantitative traits in *Cornus nuttallii* with geographic and climatic variables. Molecular diversity: linear regressions of population (A) allelic richness, (B) unbiased expected heterozygosity, and (C) observed heterozygosity with provenance latitude based on average values from five microsatellite markers for 19 populations. Quantitative traits: example linear regressions representing (D) a comparatively strong adaptive cline, (E) a highly significant but shallow adaptive cline, and (F) a comparatively weak adaptive cline, based on quantitative traits observed in a common garden of nine populations. Closed circles represent populations from the coastal portion of *C. nuttallii*'s range, while open circles represent a disjunct Idaho population. The Idaho population is plotted for comparative purposes, but was not included in regressions due to large geographic distance from the coastal range (A, B, and C), and small sample size in the common garden (D, E, and F).

in the southern geographic group (Fig. 1), only modest structuring was observed. The southern region had frequencies of 0.88 (range: 0.53–1.0) for haplotype 1, with four of the 10 populations fixed for this haplotype. Haplotype 2 was uncommon or absent in most southern populations, with the exception of the small, disjunct, southernmost population in the San Bernardino Mountains (SB). Here, the two haplotypes were present with approximately equal frequencies. The northern region showed less variation among populations, with both haplotypes present in all populations at a frequency of 0.59 (range: 0.33–0.87) for haplotype 1.

***Variation and clines in phenotypic traits***—The partitioning of variance components for phenotypic traits is presented in Table 4A. For most traits, the proportion of total variance accounted for by both population ($\sigma^2_p$) and family within population ($\sigma^2_{f(p)}$) was either low or nonsignificant. When calculable, estimated $h^2$ and $Q_{ST}$ values were low to moderate for most traits, ranging from 0.038 to 0.394 and from 0.110 to 0.629, respectively (Table 4B). Very low but significant variance among open-pollinated families within populations, rather than high variance among populations, resulted in the unusually low $h^2$ and high $Q_{ST}$ estimate for diameter. For all traits except diameter, the majority of total genetic variance resided within, rather than among populations (Table 4B).

Statistically significant clines relating first- and second-year height, diameter, and bud-flush and bud-set stage to geographic

and climatic variables are summarized in Table 5. In general, families from provenances with colder climates exhibited slower growth, earlier bud flush, and earlier bud set. No significant clines were found for $I_{-25}$, $LT_{50}$, dates of 5% and 95% growth, or duration of 5–95% growth.

## DISCUSSION

***Low molecular diversity***—*Cornus nuttallii* shows rangewide, uniformly low diversity in both nuclear microsatellite markers and cpDNA haplotypes. Nuclear microsatellite diversity is considerably lower in *C. nuttallii* than in *C. florida*. Out of eight microsatellite loci that are polymorphic in *C. florida* (Cabe and Liles, 2002), only three are polymorphic in *C. nuttallii*. For the three loci that are polymorphic in both species, $A_R = 7.33$ and $H_E = 0.511$ in *C. nuttallii*, while $A_R = 8.67$ and $H_E = 0.813$ in *C. florida* (Cabe and Liles, 2002). Because all microsatellite markers examined in *C. nuttallii* were originally developed for *C. florida*, the diversity estimates for *C. nuttallii* are likely to be biased downward. However, only two chloroplast haplotypes were detected after sequencing 3–5% of the noncoding chloroplast genome in 100 individuals, and low diversity has also been observed in *C. nuttallii* isozymes (S. J. Brunsfeld, U. Idaho, unpublished data). In combination, these three data sets provide strong evidence that *C. nuttallii* possesses low levels of molecular genetic diversity.

Because most tree species have relatively high levels of genetic diversity (Petit and Hampe, 2006), tree species with low genetic diversity are often hypothesized to have undergone a past population bottleneck (Fowler and Morris, 1977; Walter and Epperson, 2001; Grivet and Petit, 2003; Vendramin et al., 2008). *Cornus nuttallii* may have endured a prolonged bottleneck in a refugium during the Last Glacial Maximum. No private nuclear microsatellite alleles were found in any populations, and both chloroplast haplotypes span the species range, suggesting that this bottleneck occurred prior to postglacial recolonization. In contrast, if variation had been lost independently on multiple occasions following expansion into different parts of the current range, different alleles and haplotypes may have become fixed in or associated with different geographic regions (e.g., *Pinus torreyana* Parry ex Carr: Ledig and Conkle, 1983*; Thuja plicata* D. Don: O'Connell et al., 2008).

TABLE 4. Summary of variation in quantitative traits. (A) Percentage of total variance attributed to each model variance component. (B) Among-population differentiation in quantitative traits ($Q_{ST}$), heritability ($h^2$), and proportion of total genetic variance residing within ($\sigma^2_{GW}$) and among ($\sigma^2_{GA}$) populations, for phenotypic traits with significant $\sigma^2_{f(p)}$ only.

| (A) Quantitative trait | $\sigma^2_b$ (%) | $\sigma^2_p$ (%) | $\sigma^2_{b*p}$ (%) | $\sigma^2_{f(p)}$ (%) | $\sigma^2_e$ (%) |
|---|---|---|---|---|---|
| First-year height (cm) | ns | 7.1 | ns | ns | 92.9 |
| Second-year height (cm) | 14.0 | 7.0 | ns | 3.2 | 75.7 |
| Diameter (mm) | ns | 11.3 | 1.7 | 1.1 | 85.9 |
| Bud-flush stage | 6.0 | 8.4 | ns | 11.2 | 74.4 |
| Bud-set stage | 6.5 | 9.4 | 6.8 | ns | 77.3 |
| $I_{-25}$ (%) | 5.2 | 9.1 | ns | 6.7 | 79.1 |
| $LT_{50}$ (°C) | 5.8 | ns | ns | 5.7 | 88.5 |
| Date of 5% growth | ns | 2.8 | ns | ns | 97.2 |
| Date of 95% growth | 6.0 | 7.5 | ns | ns | 86.5 |
| Duration of 5–95% growth (d) | 2.2 | ns | ns | ns | 97.8 |

| (B) Quantitative trait | $\sigma^2_{GA}$ (%) | $\sigma^2_{GW}$ (%) | $h^2$ | $Q_{ST}$ |
|---|---|---|---|---|
| Second-year height (cm) | 42.5 | 57.5 | 0.121 | 0.270 |
| Diameter (mm) | 77.3 | 22.7 | 0.038 | 0.629 |
| Bud-flush stage | 19.9 | 80.1 | 0.394 | 0.110 |
| $I_{-25}$ (%) | 31.1 | 68.9 | 0.234 | 0.184 |
| $LT_{50}$ (°C) | nc | nc | 0.181 | nc |

*Notes:* Variance components—$\sigma^2_b$, variance due to block; $\sigma^2_p$, variance due population; $\sigma^2_{b*p}$, variance due to the interaction between block and population; $\sigma^2_{f(p)}$, variance due to open-pollinated family nested within population; $\sigma^2_e$, variance due to error. See Materials and Methods for full explanation of each quantitative trait. $I_{-25}$, index of injury at –25°C; $LT_{50}$, low temperature at which 50% tissue lethality occurs; nc, not calculable due to nonsignificant $\sigma^2_p$; ns, not significant at $\alpha = 0.05$.

***Phenotypic variation and local adaptation***—Although *C. nuttallii* possesses low molecular diversity, sufficient genetic variation in phenotypic traits has existed within the species to allow weak to moderate local adaptation. Estimates of among-population differentiation in phenotypic traits, $Q_{ST}$, are substantially higher than the corresponding estimates of among-population differentiation in microsatellite markers for the same nine populations ($F_{ST} = 0.090$; $R_{ST} = 0.102$) in second-year height, diameter, and $I_{-25}$, but not for bud-flush stage (Table 4B). If $Q_{ST} > F_{ST}$, this suggests that divergent selection pressures are acting upon the trait (Whitlock, 2008). However, comparisons of point estimates of $Q_{ST}$ and $F_{ST}$ are fraught with statistical difficulties (O'Hara and Merilä, 2005; Leinonen et al., 2007; Whitlock, 2008). Random sampling error may result in $Q_{ST}$ and $F_{ST}$ estimates substantially different from the true mean, even for neutral traits and loci, respectively (Whitlock, 2008). Comparing distributions of $Q_{ST}$ and $F_{ST}$ rather than point estimates may reduce some of this error, but remains statistically challenging (O'Hara and Merilä, 2005; Whitlock, 2008). The finding that $Q_{ST} > F_{ST}$ in several traits for *C. nuttallii* thus hints at a role for divergent selection and local adaptation, but is not definitive.

Stronger evidence of local adaptation in *C. nuttallii* is provided by significant clines in phenotypic traits along geographic and climatic gradients (Table 5). In general, families from colder, more northern provenances had the lowest overall growth, earliest bud flush, and earliest bud set. These trends are consistent with those typically observed in common-garden studies of temperate tree species (Howe et al., 2003). More conservative growth patterns may be adaptive in colder, harsher environments where the competitive benefits of reducing the length of the growing season to avoid spring and fall frost damage outweigh those of maximizing growth via a long growing season (Howe et al., 2003). Strong selection pressures for avoidance of damage from early fall frosts lead to earlier bud set in trees from colder provenances (Howe et al., 2003). Similarly, trees from colder provenances with less risk of premature spring bud flush triggered by warm periods in winter often have lower chilling and heat-sum requirements and experience earlier bud flush in common-garden environments (Howe et al., 2003).

Although clinal variation may result from nonadaptive processes such as secondary contact between isolated populations or isolation by distance (Vasemägi 2006), it is more likely that the patterns we observed in *C. nuttallii* are adaptive clines. There is no evidence to suggest multiple glacial refugia (see *Postglacial recolonization* below), making secondary contact of isolated populations an unlikely cause of rangewide clines. Furthermore, all clines are either exclusively or most strongly

TABLE 5. Summary of adaptive clines in *Cornus nuttallii* for selected quantitative traits, including sign of the slope (for significant regressions only), $R^2$ values, and statistical significance ($P$).

| | First-year height | | | Second-year height | | | Diameter | | | Bud-flush stage [b] | | | Bud-set stage [b] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Geoclimatic variable [a] | Sign | $R^2$ | $P$ | Sign | $R^2$ | $P$ | Sign | $R^2$ | $P$ | Sign | $R^2$ | P | Sign | $R^2$ | $P$ |
| Latitude (°N) | – | **0.1868** | **0.0001** | | 0.1050 | 0.0052 | – | **0.2316** | **<0.0001** | – | **0.1709** | **0.0003** | | 0.0202 | 0.2301 |
| Longitude (°W) | | 0.0590 | 0.0383 [c] | | 0.1603 | 0.0004 | | 0.0480 | 0.0625 | | 0.0306 | 0.1390 | | 0.0003 | 0.8849 |
| Elevation (m a.s.l.) | | 0.0736 | 0.0203 [c] | | 0.0658 | 0.0284 [c] | | 0.0426 | 0.0798 | | 0.0816 | 0.0173 [c] | | 0.0146 | 0.3083 |
| MAT (°C) | + | **0.2054** | **<0.0001** | + | **0.1255** | **0.0021** | + | **0.3276** | **<0.0001** | + | **0.2015** | **<0.0001** | | 0.0752 | 0.0189 [c] |
| MWMT (°C) | | 0.0729 | 0.0209 [c] | + | **0.1143** | **0.0034** | + | **0.1246** | **0.0022** | + | **0.0985** | **0.0069** | | 0.0034 | 0.6242 |
| MCMT (°C) | + | **0.2102** | **<0.0001** | | 0.0672 | 0.0268 [c] | + | **0.3203** | **<0.0001** | + | **0.2073** | **<0.0001** | + | **0.1269** | **0.0020** |
| MAP (mm) | | 0.0603 | 0.0363 [c] | – | **0.1395** | **0.0011** | | 0.0265 | 0.1686 | | 0.0099 | 0.4027 | | 0.0000 | 0.9958 |
| MSP (mm) | – | **0.1299** | **0.0017** | – | **0.1207** | **0.0026** | – | **0.1465** | **0.0008** | – | **0.0923** | **0.0090** | | 0.0023 | 0.6879 |
| FFP (days) | + | **0.2052** | **<0.0001** | + | **0.0896** | **0.0101** | + | **0.3352** | **<0.0001** | + | **0.1755** | **0.0002** | + | **0.1463** | **0.0008** |
| GDD | + | **0.1810** | **0.0002** | + | **0.1594** | **0.0005** | + | **0.2893** | **<0.0001** | + | **0.1595** | **0.0005** | | 0.0272 | 0.1632 |

*Notes:* No significant regressions were found for $I_{-25}$ (index of injury at –25°C), $LT_{50}$ (low temperature at which 50% tissue lethality occurs), dates of 5% and 95% growth, or duration of 5–95% growth (data not shown).

[a] See Table 1 for abbreviations of geographic and climatic variables.

[b] Note that a higher bud-flush and bud-set stages indicate that the phenological event occurred later in the year.

[c] No longer significant after sequential Bonferonni correction for multiple comparisons (trait-wide $\alpha = 0.05$) (Rice, 1989).

associated with climatic gradients. Stronger associations with geographic rather than climatic gradients would be expected if clines resulted from isolation by distance. Finally, the observed clines correspond to those documented in other temperate tree species and have simple adaptive explanations. Together, these pieces of evidence suggest that adaptation to local climate is the most likely explanation for the rangewide establishment and maintenance of these phenotypic clines.

Despite evidence of climatic adaptation, *C. nuttallii* is not as strongly locally adapted as many other temperate and boreal tree species. Estimated values of $Q_{ST}$ and ratios of $Q_{ST}$ to $F_{ST}$ for traits other than diameter are lower than those typically observed for similar traits in other trees (Howe et al., 2003; Savolainen et al., 2007). Significant adaptive clines also have lower $R^2$ values (Table 5) and shallower slopes (Fig. 2) than those typical of many trees (e.g., Campbell, 1979; Rehfeldt et al., 1999; Mimura and Aitken, 2007; Savolainen et al., 2007). Furthermore, adaptive clines were absent in fall cold-hardiness traits ($I_{-25}$, $LT_{50}$) and timing of height growth, which are often under strong divergent selection in other species (Savolainen et al., 2007).

***Postglacial recolonization***—*Cornus nuttallii* likely survived in a single southern refugium during the Last Glacial Maximum. The decline in nuclear-microsatellite diversity with latitude is consistent with leading edge, postglacial recolonization (Cwynar and Macdonald, 1987; Hewitt, 1993; Allen et al., 1996). The leading edge hypothesis suggests that populations expanded northward from a southern refugium via long-distance dispersal, facilitated by individuals along the leading edge of recolonization. Stochastic processes occurring during such a range expansion event could have resulted in the decrease in genetic variation in newly founded northern populations. Only very weak structuring of cpDNA into southern and northern haplotypes was observed on either side of the Soltis line (Soltis et al., 1997; Brunsfeld et al., 2007), providing little evidence that *C. nuttallii* exhibits the same pattern of north–south haplotype structuring that has been observed in several other sympatric species.

Relatively wet conditions during the Last Glacial Maximum are hypothesized to have created a large refugium for mesic temperate forests south of glaciation in the Pacific Northwest (Brunsfeld et al., 2001). Major proposed refugia within this region include the Coastal Mountains, the Columbia River basin along the Idaho–Oregon border, and the Rocky Mountains of Montana (Shafer et al., 2010). Within the present range of *C. nuttallii*, smaller refugia have also been proposed for temperate terrestrial biota in the Siskiyou–Klamath Mountains near the California–Oregon border, the Olympic Peninsula in Washington, and the Clearwater River basin in northern Idaho (Shafer et al., 2010). Populations from the locations of all proposed refugia within *C. nuttallii*'s current range were included in this study (Fig. 1), yet a precise refugial location cannot be identified given the observed lack of chloroplast diversity. However, the significant decline in allelic richness, expected heterozygosity, and observed heterozygosity with latitude in nuclear microsatellite loci (Fig. 2) suggests that the refugium may have been well toward the southern portion of the species distribution.

Populations sampled in southern California (including the disjunct SB population) and northern Idaho (CL) were likely established by postglacial dispersal. No mesic forest refugium has been established from fossil data in California in either the Sierra Nevada or the southern Cascade Mountains (Brunsfeld et al., 2001). The Clearwater River basin in northern Idaho is a proposed glacial refugium for other species (Shafer et al., 2010), but the Idaho population of *C. nuttallii* has lower nuclear molecular diversity than other populations (Table 3) and no private alleles or haplotypes, suggesting that this population was established via more recent dispersal (Brunsfeld et al., 2001; Carstens et al., 2005). We cannot ascertain if this putative dispersal event occurred from a northern or southern source, as the Idaho population is fixed for haplotype 1, which is found in all other populations (Fig. 1).

*Cornus nuttallii* likely possesses the ability for long-distance dispersal of the type necessary to establish disjunct populations because its seeds are dispersed by animals such as migratory birds (e.g., American robin, *Turdus migratorius* L.: Willson, 1994). Low $F_{ST}$ estimates also suggest that long-distance dispersal plays an important role in shaping the population genetic structure of *C. nuttallii*, although the assumptions required to interpret $F_{ST}$ as an approximation of gene flow are unlikely

to be met (Whitlock and McCauley, 1999). Additionally, the significant isolation by distance in the continuous portion of the species range suggests that gene flow between neighboring populations occurs more frequently than gene flow over longer distances.

Though we suggest a relatively simple phylogeography for *C. nuttallii*, there is growing evidence to suggest that the phylogeographic history of most species in northwestern North America is more complicated than had previously been hypothesized (Shafer et al., 2010). Many species may have survived in multiple refugia, including in previously unknown refugia within and along the edges of ice sheets, or in multiple subrefugia within larger refugial regions (Shafer et al., 2010). However, leading-edge recolonization from a single southern refugium that faced a severe population bottleneck remains the most parsimonious explanation for the available data for *C. nuttallii*, and there is no evidence to suggest a more complex postglacial recolonization pattern. In this respect, *C. nuttallii* may resemble *Thuja plicata*, another western North American tree species with both a coastal and disjunct interior range that is proposed to have survived in a single southern refugium (O'Connell et al., 2008). Nonetheless, our phylogeographic conclusions are both supported by and weakened by the low level of diversity found in both chloroplast haplotypes and nuclear microsatellite markers. We acknowledge that if more diversity is found in some markers in the future, stronger phylogeographic signals may be detected, and a more complex pattern of postglacial recolonization may be revealed.

***Conservation and seed-transfer implications***—As climate change progresses, tree populations will need to adapt to new conditions, migrate to track changing climates, or tolerate changes through phenotypic plasticity to avoid maladaptation and potential extirpation (Aitken et al., 2008). Low genetic diversity may lower *C. nuttallii*'s adaptive capacity relative to the average capacity of other tree species. However, this species has demonstrated some capacity to adapt since the Last Glacial Maximum as evidenced by genetic clines in adaptive traits. *Cornus nuttallii* may be more of an adaptive generalist than many other sympatric western North American trees that are strongly locally adapted (Howe et al., 2003; Savolainen et al., 2007) and may have the capacity to tolerate changes through phenotypic plasticity. We were unable to evaluate the phenotypic plasticity of this species in our single common garden environment. However, the relatively flat quantitative genetic clines and lack of strong molecular genetic differentiation suggest seed transfer for restoration or reforestation will result in smaller risks of maladaptation than predicted for species with stronger population differentiation and steeper clines. Transfers should favor the collection of seed in warmer climates for planting in colder locations (higher latitude or elevation) to assist the migration of genotypes preadapted to future conditions within the present species range (Aitken et al., 2008).

The small, disjunct Idaho population (CL) has the lowest diversity and highest genetic differentiation (Table 3) and is therefore the population with highest conservation priority. Populations at the lagging edge of migration in southern California are also of high priority for genetic conservation because those populations will be at greatest risk of maladaptation in a changing climate (Davis and Shaw, 2001; Aitken et al., 2008).

## LITERATURE CITED


AITKEN, S. N., S. YEAMAN, J. A. HOLLIDAY, T. L. WANG, AND S. CURTIS-MCLANE. 2008. Adaptation, migration or extirpation: Climate change outcomes for tree populations. *Evolutionary Applications* 1: 95–111.

ALLEN, G. A., J. A. ANTOS, A. C. WORLEY, T. A. SUTTILL, AND R. J. HEBDA. 1996. Morphological and genetic variation in disjunct populations of the avalanche lily *Erythronium montanum. Canadian Journal of Botany* 74: 403–412.

BOWER, A. D., AND S. N. AITKEN. 2006. Geographic and seasonal variation in cold hardiness of whitebark pine. *Canadian Journal of Forest Research* 36: 1842–1850.

BROWN, J. S., AND N. B. PAVLOVIC. 1992. Evolution in heterogeneous environments: Effects of migration on habitat specialization. *Evolutionary Ecology* 6: 360–382.

BRUNSFELD, S. J., T. R. MILLER, AND B. C. CARSTENS. 2007. Insights into the biogeography of the Pacific Northwest of North America: Evidence from the phylogeography of *Salix melanopsis*. *Systematic Botany* 32: 129–139.

BRUNSFELD, S. J., J. SULLIVAN, D. E. SOLTIS, AND P. E. SOLTIS. 2001. Comparative phylogeography of northwestern North America: A synthesis. *In* J. Silvertown and J. Antonovics [eds.], Integrating ecology and evolution in a spatial context, 319–339. Blackwell Science, Oxford, UK.

CABE, P. R., AND J. S. LILES. 2002. Dinucleotide microsatellite loci isolated from flowering dogwood (*Cornus florida* L.). *Molecular Ecology Notes* 2: 150–152.

CAMPBELL, R. K. 1979. Genecology of Douglas-fir in a watershed in the Oregon Cascades. *Ecology* 60: 1036–1050.

CARSTENS, B. C., S. J. BRUNSFELD, J. R. DEMBOSKI, J. M. GOOD, AND J. SULLIVAN. 2005. Investigating the evolutionary history of the Pacific Northwest mesic forest ecosystem: Hypothesis testing within a comparative phylogeographic framework. *Evolution* 59: 1639–1652.

CORRIVEAU, J. L., AND A. W. COLEMAN. 1988. Rapid screening method to detect potential biparental inheritance of plastid DNA and results for over 200 angiosperm species. *American Journal of Botany* 75: 1443–1458.

CWYNAR, L. C., AND G. M. MACDONALD. 1987. Geographical variation of lodgepole pine in relation to population history. *American Naturalist* 129: 463–469.

DAVIS, M. B., AND R. G. SHAW. 2001. Range shifts and adaptive responses to quaternary climate change. *Science* 292: 673–679.

DOYLE, J. J., AND J. L. DOYLE. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11–15.

ENGLAND, P. R., G. H. R. OSLER, L. M. WOODWORTH, M. E. MONTGOMERY, D. A. BRISCOE, AND R. FRANKHAM. 2003. Effects of intense versus diffuse population bottlenecks on microsatellite genetic diversity and evolutionary potential. *Conservation Genetics* 4: 595–604.

EXCOFFIER, L., P. E. SMOUSE, AND J. M. QUATTRO. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial-DNA restriction data. *Genetics* 131: 479–491.

FLINT, H. L., B. R. BOYCE, AND D. J. BEATTIE. 1967. Index of injury: A useful expression of freezing injury to plant tissues as determined by the electrolytic method. *Canadian Journal of Plant Science* 47: 229–230.

FOWLER, D. P., AND R. W. MORRIS. 1977. Genetic diversity in red pine: Evidence of low genetic heterozygosity. *Canadian Journal of Forest Research* 7: 343–347.

GONZÁLEZ-MARTÍNEZ, S. C., K. V. KRUTOVSKY, AND D. B. NEALE. 2006. Forest-tree population genomics and adaptive evolution. *New Phytologist* 170: 227–238.

GRIVET, D., AND R. J. PETIT. 2003. Chloroplast DNA phylogeography of the hornbeam in Europe: Evidence for a bottleneck at the outset of postglacial colonization. *Conservation Genetics* 4: 47–56.

HAMILTON, M. B. 1999. Four primer pairs for the amplification of chloroplast intergenic regions with intraspecific variation. *Molecular Ecology* 8: 521–523.

HANNERZ, M., S. N. AITKEN, J. N. KING, AND S. BUDGE. 1999. Effects of genetic selection for growth on frost hardiness in western hemlock. *Canadian Journal of Forest Research* 29: 509–516.

HEWITT, G. M. 1993. Postglacial distribution and species substructures: Lessons from pollen insects and hybrid zones. *In* D. R. Lees and D. Edwards [eds.], Evolutionary patterns and processes, 97–123. Academic Press, London, UK.

HOWE, G. T., S. N. AITKEN, D. B. NEALE, K. D. JERMSTAD, N. C. WHEELER, AND T. H. H. CHEN. 2003. From genotype to phenotype: Unraveling the complexities of cold adaptation in forest trees. *Canadian Journal of Botany* 81: 1247–1266.

KLINKA, K., J. WORRALL, L. SKODA, AND P. VARGA. 2000. The distribution and synopsis of ecological and silvical characteristics of tree species of British Columbia's forests. Canadian Cartographics, Coquitlam, British Columbia, Canada.

LANDE, R. 1995. Mutation and conservation. *Conservation Biology* 9: 782–791.

LEDIG, F. T., AND M. T. CONKLE. 1983. Gene diversity and genetic structure in a narrow endemic, Torrey pine (*Pinus torreyana* Parry ex Carr). *Evolution* 37: 79–85.

LEINONEN, T., R. B. O'HARA, J. M. CANO, AND J. MERILÄ. 2007. Comparative studies of quantitative trait and neutral marker divergenece: A meta-analysis. *Journal of Evolutionary Biology* 21: 1–17.

MANTEL, N. 1967. Detection of disease clustering and a generalized regression approach. *Cancer Research* 27: 209–220.

MEAGHER, M. D., AND R. S. HUNT. 1998. Early height growth of western white pine provenances in British Columbia plantations. *Western Journal of Applied Forestry* 13: 47–53.

MIMURA, M., AND S. N. AITKEN. 2007. Adaptive gradients and isolation-by-distance with postglacial migration in *Picea sitchensis. Heredity* 99: 224–232.

O'CONNELL, L. M., K. RITLAND, AND S. L. THOMPSON. 2008. Patterns of post-glacial colonization by western redcedar (*Thuja plicata*, Cupressaceae) as revealed by microsatellite markers. *Botany* 86: 194–203.

O'HARA, R. B., AND J. MERILÄ. 2005. Bias and precision in $Q_{ST}$ estimates: Problems and some solutions. *Genetics* 171: 1331–1339.

PEAKALL, R., AND P. E. SMOUSE. 2006. GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* 6: 288–295.

PETIT, R. J., AND A. HAMPE. 2006. Some evolutionary consequences of being a tree. *Annual Review of Ecology, Evolution and Systematics* 37: 187–214.

REHFELDT, G. E., R. J. HOFF, AND R. J. STEINHOFF. 1984. Geographic patterns of genetic variation in *Pinus monticola. Botanical Gazette* 145: 229–239.

REHFELDT, G. E., AND W. R. WYKOFF. 1981. Periodicity in shoot elongation among populations of *Pinus contorta* from the northern Rocky Mountains. *Annals of Botany* 48: 371–377.

REHFELDT, G. E., C. C. YING, D. L. SPITTLEHOUSE, AND D. A. HAMILTON. 1999. Genetic responses to climate in *Pinus contorta*: Niche breadth, climate change, and reforestation. *Ecological Monographs* 69: 375–407.

RICE, W. R. 1989. Analyzing tables of statistical tests. *Evolution* 43: 223–225.

ROUSSET, F. 1997. Genetic differentiation and estimation of gene flow from *F*-statistics under isolation by distance. *Genetics* 145: 1219–1228.

ROZEN, S., AND H. J. SKALETSKY. 2000. Primer on the WWW for general users and for biologist programmers. *In* S. Krawetz and S. Misener [eds.], Bioinformatics methods and protocols: Methods in molecular biology, 365–386. Humana Press, Totowa, New Jersey, USA.

SAVOLAINEN, O., T. PYHÄJÄRVI, AND T. KNÜRR. 2007. Gene flow and local adaptation in trees. *Annual Review of Ecology, Evolution and Systematics* 38: 595–619.

SHAFER, A. B. A., C. I. CULLINGHAM, S. D. CÔTÉ, AND D. W. COLTMAN. 2010. Of glaciers and refugia: A decade of study sheds new light on the phylogeography of northwestern North America. *Molecular Ecology* 19: 4589–4621.

SHAW, J., E. B. LICKEY, J. T. BECK, S. B. FARMER, W. S. LIU, J. MILLER, K. C. SIRIPUN, ET AL. 2005. The tortoise and the hare II: Relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. *American Journal of Botany* 92: 142–166.

SLATKIN, M. 1995. A measure of population subdivision based on microsatellite allele frequencies. *Genetics* 139: 1463.

SOLTIS, D. E., M. A. GITZENDANNER, D. D. STRENGE, AND P. S. SOLTIS. 1997. Chloroplast DNA intraspecific phylogeography of plants from the Pacific Northwest of North America. *Plant Systematics and Evolution* 206: 353–373.

SPITZE, K. 1993. Population structure in *Daphnia obtusa*: Quantitative genetic and allozymic variation. *Genetics* 135: 367–374.

SQUILLACE, A. E. 1974. Genetic correlations among offspring from open-pollinated forest trees. *Silvae Genetica* 23: 149–156.

TAMURA, K., J. DUDLEY, M. NEI, AND S. KUMAR. 2007. MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* 24: 1596–1599.

VASEMÄGI, A. 2006. The adaptive hypothesis of clinal variation revisited: Single-locus clines as a result of spatially restricted gene flow. *Genetics* 173: 2411–2414.

VENDRAMIN, G. G., B. FADY, S. C. GONZÁLEZ-MARTÍNEZ, F. S. HU, I. SCOTTI, F. SEBASTIANI, A. SOTO, ET AL. 2008. Genetically depauperate but widespread: The case of an emblematic Mediterranean pine. *Evolution* 62: 680–688.

WADDELL, K. L., AND B. HISEROTE. 2005. The PNW-FIA integrated database user guide and documentation: Version 2.0. Pacific Northwest Research Station internal publication, Portland, Oregon, USA.

WAKASUGI, T., M. SUGITA, T. TSUDZUKI, AND M. SUGIURA. 1998. Updated gene map of tobacco chloroplast DNA. *Plant Molecular Biology Reporter* 16: 231–241.

WALTER, R., AND B. K. EPPERSON. 2001. Geographic pattern of genetic variation in *Pinus resinosa*: Area of greatest diversity is not the origin of postglacial populations. *Molecular Ecology* 10: 103–111.

WANG, T., A. HAMANN, D. L. SPITTLEHOUSE, AND S. N. AITKEN. 2006. Development of scale-free climate data for western Canada for use in resource management. *International Journal of Climatology* 26: 383–397.

WEIR, B. S., AND C. C. COCKERHAM. 1984. Estimating *F*-statistics for the analysis of population structure. *Evolution* 38: 1358–1370.

WHITLOCK, M. C. 2008. Evolutionary inference from $Q_{ST}$. *Molecular Ecology* 17: 1885–1896.

WHITLOCK, M. C., AND D. E. MCCAULEY. 1999. Indirect measures of gene flow and migration: $F_{ST} \neq 1/(4Nm+1)$. *Heredity* 82: 117–125.

WILLSON, M. F. 1994. Fruit choice by captive American robins. *Condor* 96: 494–502.

XIANG, Q. Y. J., S. J. BRUNSFELD, D. E. SOLTIS, AND P. S. SOLTIS. 1996. Phylogenetic relationships in *Cornus* based on chloroplast DNA restriction sites: Implications for biogeography and character evolution. *Systematic Botany* 21: 515–534.

XIANG, Q. Y. J., D. T. THOMAS, W. H. ZHANG, S. R. MANCHESTER, AND Z. MURRELL. 2006. Species level phylogeny of the genus *Cornus* (Cornaceae) based on molecular and morphological evidence: Implications for taxonomy and Tertiary intercontinental migration. *Taxon* 55: 9–30.