

WILEY

Ecological Genetics and Seed Transfer Guidelines for *Pinus albicaulis* (Pinaceae)

Author(s): Andrew D. Bower and Sally N. Aitken

Source: *American Journal of Botany*, Jan., 2008, Vol. 95, No. 1 (Jan., 2008), pp. 66-76

Published by: Wiley

Stable URL: <https://www.jstor.org/stable/27733392>

JSTOR is a not-for-profit service that helps scholars, researchers, and students discover, use, and build upon a wide range of content in a trusted digital archive. We use information technology and tools to increase productivity and facilitate new forms of scholarship. For more information about JSTOR, please contact support@jstor.org.

Your use of the JSTOR archive indicates your acceptance of the Terms & Conditions of Use, available at <https://about.jstor.org/terms>



Wiley is collaborating with JSTOR to digitize, preserve and extend access to *American Journal of Botany*

JSTOR

ECOLOGICAL GENETICS AND SEED TRANSFER GUIDELINES FOR *PINUS ALBICAULIS* (PINACEAE)¹

ANDREW D. BOWER² AND SALLY N. AITKEN

Centre for Forest Conservation Genetics, Department of Forest Sciences, University of British Columbia, 3401-2424 Main Mall, Vancouver, British Columbia V6T 1Z4 Canada

Whitebark pine (*Pinus albicaulis* Engelm.) has greatly declined throughout its range as a result of introduced disease, fire suppression, and other factors, and climate change is predicted to accelerate this decline. Restoration is needed; however, no information regarding the degree of local adaptation is available to guide these efforts. A seedling common-garden experiment was employed to assess genetic diversity and geographic differentiation (Q_{ST}) of whitebark pine for traits involved in growth and adaptation to cold and to determine climatic variables revealing local adaptation. Seedlings from 48 populations were grown for two years and measured for height increment, biomass, root to shoot ratio, date of needle flush, fall and spring cold injury, and survival. Significant variation was observed among populations for most traits. The Q_{ST} was low (0.07–0.14) for growth traits and moderate (0.36–0.47) for cold adaptation related traits, but varied by region. Cold adaptation traits were strongly correlated with mean temperature of the coldest month of population origins, while growth traits were generally correlated with growing season length. We recommend that seed transfer for restoration favor seed movement from milder to colder climates to a maximum of 1.9°C in mean annual temperature in the northern portion of the species range, and 1.0°C in the U. S. Rocky Mountains to avoid maladaptation to current conditions yet facilitate adaptation to future climates.

Key words: genetic variation; geographic differentiation; local adaptation; *Pinus albicaulis*; quantitative traits; seed transfer; whitebark pine; white pine blister rust.

Movement of seeds from their collection site to other environments within a species range for reforestation or restoration may increase the risk of maladaptation (Campbell, 1979). Reduced growth or mortality resulting from maladaptation could reduce the success of restoration projects, and gene flow from maladapted planted trees into adjacent native populations could negatively affect adaptation to local conditions (McKay et al., 2005). However, the planting of individuals adapted to new environmental conditions, e.g., a warmer climate, could be a method to facilitate migration and provide a source of genotypes well adapted to local populations. Seed transfer should be guided by natural levels of genetic variation and local adaptation in quantitative traits specific to the species in question (Morgenstern, 1996; Hufford and Mazer, 2003; McKay et al., 2005). Understanding genetic structure is also necessary for managing breeding programs, evaluating conservation of genetic resources, and predicting the possible effects of climate change (St. Clair et al., 2005).

The ranges of many tree species are predicted to shift higher in latitude and elevation as a result of climate change (Davis and Shaw, 2001; Hamann and Wang, 2006). However, at a local scale, projected vegetation responses include a combination of eleva-

tional, aspect, and microsite adjustments because the location of suitable conditions for each taxon shifts within a region (Bartlein et al., 1997). The potential impacts of predicted warming underscore the importance of understanding genetic structure and adaptation of populations to their local environment. For species threatened by pests and diseases in addition to climate change, minimizing maladaptation may mean the difference between establishing or maintaining viable populations and local extirpation.

Whitebark pine (*Pinus albicaulis* Engelm., Pinaceae) is a high elevation, five-needle pine, and the only North American member of the stone pines (*Pinus* subsection *Cembrae*) (Arno and Hoff, 1989; Price et al., 1998; but see Gernandt et al., 2005). Although of little commercial value, it has tremendous ecological value and is considered a keystone species (Tomback et al., 2001). The large, wingless seeds of whitebark pine are an important food source for the Clark's nutcracker (*Nucifraga columbiana* Wilson), which is its primary dispersal agent and mutualist (Tomback, 1978; Hutchins and Lanner, 1982; Lanner, 1982; Tomback, 1982). However, whitebark pine is in decline throughout most of its range from a synergism of natural and human-driven causes. Outbreaks of mountain pine beetle (*Dendroctonus ponderosae* Hopkins) and decades of fire suppression have led to mortality and successional replacement by shade-tolerant species. However, the greatest agent driving the current decline is the introduced disease white pine blister rust (caused by the fungus *Cronartium ribicola* J. C. Fisch. ex Rabh.). Scientists agree that whitebark pine ecosystems require immediate restoration to reduce the effects of fire exclusion and blister rust (McCool and Freimund, 2001). Silvicultural techniques can be used to encourage natural regeneration, but in stands with a compromised seed source or those that need to be regenerated quickly, planting seedlings (if available) is the suggested restoration practice (Hoff et al., 2001), using blister-rust-resistant seedlings when they are available. There is a widespread need for restoration and often a limited supply of seed for whitebark pine, thus geographic guidelines on seed transfer are needed for restoration and conservation of this species.

¹ Manuscript received 21 September 2006; revision accepted 8 November 2007.

The authors thank the USDA Forest Service regions one, five, and six; the British Columbia Ministry of Forests; E. C. Manning and Tweedsmuir Provincial Parks of British Columbia; and B. Brett of Snowline Ecological Consulting, Whistler, B.C. for seed. Many people provided assistance to this project, including D. Kolotelo, J. Tuytel, C. Chourmouzis, D. Watson, K. Keir, M. Harrison, D. Szohner, P. Smets, J. Krakowski, S. Trehearne, and all of the members of the Aitken laboratory at UBC. Climate data were provided by Drs. T. Wang and G. Rehfeldt. Funding for this study came from the British Columbia Forestry Investment Account through the Forest Genetics Council of B.C. to the Centre for Forest Conservation Genetics at UBC. Thank you to Drs. A. Yanchuk, M. Whitlock, J. Whitton, Y. El-Kassaby, S. Graham, D. Tomback, B. St. Clair, and an anonymous reviewer for their helpful comments on earlier drafts of this manuscript.

² Author for correspondence (e-mail: andrew.bower@comcast.net)

Genetic variation and population differentiation have been assessed in whitebark pine using molecular markers and monoterpenes (Yandell, 1992; Jorgensen and Hamrick, 1997; Bruederle et al., 1998; Stuart-Smith, 1998; Rogers et al., 1999; Krakowski et al., 2003), and results have indicated average to above average expected heterozygosity compared to other pines in general [Ledig, 1998]. Population differentiation in whitebark pine was reported to be low to moderate in all studies (F_{ST} or $G_{ST} < 0.09$) (Table 1), with significant evidence of inbreeding (F_{is} significantly greater than zero). Populations in the northern (western British Columbia), eastern (Rocky Mountains), and southern regions of the species range (California and Oregon) are differentiated for monoterpenes (Zavarin et al., 1991), isozymes (Yandell, 1992) and organelle DNA (Richardson et al., 2002b). However, levels of genetic variation and population differentiation in phenotypic traits potentially involved in local adaptation in whitebark pine have not previously been determined.

In this study, we analyze geographic variation and genetic differentiation in phenotypic seedling traits in a common-garden experiment in whitebark pine and evaluate degree of local adaptation to climate for the purpose of developing seed transfer recommendations and predicting the ability of whitebark pine to adapt to climate change.

MATERIALS AND METHODS

Sample materials—Open-pollinated seeds from 48 populations of whitebark pine from across most of the species range (Table 2, Fig. 1) were germinated in 2002 following seed stratification using the protocol described by Burr et al. (2001). Germinants were sown into individual 10 in³ (164 cm³) Ray Leach Cone-tainer super cells (Stuewe and Sons, Corvallis, Oregon, USA) for their first growing season. In November 2002, 10-mo-old seedlings were transplanted into a raised nursery bed common garden in Vancouver, British Columbia (49°13'N, 123°6'W) and grown for two growing seasons. Seedlings were planted in an incomplete block alpha design (Patterson and Williams, 1976) with 12 replications, and 10 four-tree by four-tree incomplete blocks within replications. Each replication contained 160 test trees, with populations represented by 1–18 families (mean 7.9, SE 0.37), with each family usually represented once per replication. Because temperatures in Vancouver are higher than those in the native environment, two temperature treatments were imposed:

eight replications had ambient soil temperature (ambient treatment) and the remaining four replications (cold treatment) had cooled water pumped through hoses buried approximately 25 cm below the surface, which kept soil temperature consistently ~8°C cooler during the warmest part of the day. Populations that were represented in fewer than half of the replications (i.e., <4 in the ambient or <2 in the cold treatment) because of mortality were excluded from the analysis. The final data set included 40 populations in the ambient treatment and 37 in the cold treatment, with 33 populations common to both treatments (Table 2). The AlphaPlus program (Mann, 1996) was used to design the planting layout and assign seedlings randomly within replications. Seedlings were planted at 9.5 × 10 cm spacing, with one row of buffer trees surrounding each raised nursery bed for which data were not collected. They were kept well watered and were fertilized and weeded as needed to provide conditions optimal for growth for most temperate conifers. Timing of initiation of growth in the spring was observed for 2003 and 2004, and at the end of the 2004 growing season, survival, height growth, aboveground and belowground oven-dry biomass of seedlings were measured on all replications. Artificial freeze testing was performed on 5-mm needle segments from all seedlings in the ambient treatment in three replications in the fall of 2003 and four replications in the spring of 2004. The electrolyte leakage method was used to quantify cold injury. Details of cold hardiness testing are given in Bower and Aitken (2006).

The final data set contained 10 quantitative variables; data from all trees in both temperature treatments were available for third-year height increment, root biomass, shoot biomass, total biomass, root to shoot ratio, date of needle flush in 2003 and 2004 (Table 3). Measurements of fall and spring cold injury were available from the ambient treatment only. In addition, the percentage survival in each soil temperature treatment was tested for treatment effects.

Data analysis—SAS version 8 (SAS Institute, 1999) was used for all statistical analyses. Preliminary analysis showed an increase in variability of residuals with an increase in predicted values, so a natural-log transformation was applied to height increment, root, shoot, and total biomass, and root to shoot ratio for all analyses, which helped to equalize variance. For testing for differences between soil temperature treatments and genotype-by-environment interactions in the quantitative traits, PROC MIXED was used with the following model for populations included in both treatments:

$$y_{ijklmn} = \mu + t_i + r(t)_{ij} + b(rt)_{ijk} + p_l + pt_{il} + pr(t)_{jli} + f(p)_{lm} + e_{ijklmn}, \quad (\text{Eq. 1})$$

where y_{ijklmn} is the observed value for tree n in family m within population l in incomplete block k in rep j in soil temperature i , μ is the overall mean, t_i is the effect of temperature i , $r(t)_{ij}$ is the effect of rep j nested within temperature i , $b(rt)_{ijk}$ is the effect of incomplete block k nested within rep j within temperature i , p_l is the effect of population l , pt_{il} is the interaction of temperature i and population l , $pr(t)_{jli}$ is the interaction of population l and rep j within temperature i , $f(p)_{lm}$ is the effect of family m nested within population l , and e_{ijklmn} Temperature, population, and population-by-temperature interaction were considered fixed, while all other effects were considered random. The same model was used to analyze each geographic region separately. Population

TABLE 1. Reported values of genetic differentiation for whitebark pine (*Pinus albicaulis*) and other stone pine (*Pinus* subsection *Cembrae*) species.

Populations	Species	Area	F_{ST} or G_{ST}	Reference
14	<i>P. albicaulis</i>	BC, ID, MT, OR	0.075	A. Bower unpublished manuscript
30	<i>P. albicaulis</i>	USA rangewide and northern AB	0.034	Jorgensen and Hamrick 1997
14	<i>P. albicaulis</i>	USA Great Basin	0.088	Yandell 1992
29	<i>P. albicaulis</i>	Canadian Rockies	0.062	Stuart-Smith 1998
17	<i>P. albicaulis</i>	British Columbia	0.061	Krakowski et al. 2003
18	<i>P. albicaulis</i>	Rangewide	0.046 ^a	Richardson et al. 2002b
8	<i>P. sibirica</i>	Russia	~0.042	Goncharenko et al. 1993b
11	<i>P. sibirica</i>	Russia	0.025	Krutovskii et al. 1995
	<i>P. koraiensis</i>	Coastal Russia	0.016	Potenko and Velikov 2001
19	<i>P. koraiensis</i>	Russia	0.015	Potenko and Velikov 1998
3	<i>P. koraiensis</i>	Russian far east	0.040	Krutovskii et al. 1995
5	<i>P. pumila</i>	Russia	0.043	Goncharenko et al. 1993a
3	<i>P. pumila</i>	Kamchatka penn., Russia	0.021	Krutovskii et al. 1995
18	<i>P. pumila</i>	Japan	0.170	Tani et al. 1996
5	<i>P. cembra</i>	Alps and eastern Carpathians, Ukraine	0.040	Belokon et al. 2005

Notes: AB = Alberta, BC = British Columbia, Canada; ID = Idaho, MT = Montana, USA.

^a Φ_{ST} from cpDNA microsatellite data

TABLE 2. *Pinus albicaulis* populations, number of seedlings tested, geographic and climatic data.

Site no.	Region	Name	No. trees		Lat. °N	Long. °W	Elev. (m)	MAT (°C)	MTWM (°C)	MTCM (°C)	FFP (d)	SH:M
			Ambient	Cold								
1	N	Serb Creek	5	10	54.71	127.57	1385	0.7	11.4	-10.7	52	32.7
2	N	Hunters Basin	13	29	54.53	127.18	1446	0.3	11.1	-11	48	34.8
3	N	Morice Lake	2	—	54.04	127.48	1231	0.6	11.3	-11.1	31	44.9
4	N	Kimsquit river	1	—	53.19	127.18	900	3	12.5	-7.2	83	32.9
5	N	Heckman Pass	6	—	52.52	125.82	1526	0	9.9	-10.6	58	46.8
6	N	Perkins Peak	3	1	51.83	125.05	1916	-1.8	7.9	-11.5	35	31.9
7	N	Jesamond	42	19	51.27	121.87	1846	0.9	11.4	-9.1	45	45
8	N	Lime Mtn.	19	13	51.10	121.67	1900	0.5	11.1	-9.4	39	52.9
9	N	Darcy	46	9	50.53	122.58	1800	0.5	11.7	-9.8	46.1	45.3
10	N	Blackcomb	57	40	50.10	122.90	1908	0.6	10	-7.5	43.7	22.4
11	N	Thynne Mtn.	20	1	49.71	120.92	1785	1.9	12.7	-7.7	48.8	27.4
12	N	Manning Park	93	40	49.10	120.67	2000	0.3	10.8	-8.6	43.8	48.1
13	N	Baldy	14	—	49.17	119.25	2154	1.2	12.1	-8.6	39.6	37.5
14	R	Copper Butte	11	9	48.70	118.46	2185	-0.5	10.4	-10.2	48.2	30.7
15	R	Colville	6	4	48.66	118.46	2154	-0.1	10.7	-10.1	49	32.5
16	R	Snow Peak	—	7	48.58	118.48	2185	0.5	11.2	-9.7	48.7	35.6
17	R	Salmo Mtn.	13	11	48.97	117.10	2092	0	10.7	-9.3	61.2	21.2
18	R	Hooknose Mtn.	—	2	48.94	117.43	2215	0.5	11.5	-8.9	54	28.8
19	R	Farnham Ridge	31	26	48.84	116.51	1846	1.5	12.4	-8.3	70.6	35.4
20	R	North Baldy	—	7	48.55	117.16	1877	2.6	13.9	-7	89	41.6
21	R	Lunch Peak	37	14	48.38	116.19	1846	2.1	12.4	-6.6	84	23.9
22	R	Our Lake	6	13	47.84	112.81	2277	0.2	11.4	-9.6	31.3	35.1
23	R	Sheep Shed	22	19	47.52	112.80	2154	1	12.3	-8.9	33.4	43.5
24	R	Granite Butte	19	8	46.87	112.47	2338	0.5	12	-9.1	32.8	47.1
25	R	Blacklead Mtn.	23	41	46.64	114.86	2062	1.2	12.3	-8.1	32.1	26.2
26	R	Gospel Peak	22	16	45.63	115.95	2154	1	11.9	-8.3	26.4	33.9
27	R	Heavens Gate	10	3	45.38	116.51	2154	1.2	12.3	-8.3	40.6	49.2
28	R	Mudd Ridge	23	15	45.90	113.45	2400	0.2	11.8	-9.5	17.5	29
29	R	Quartz Hill	27	44	45.71	112.93	2646	-0.8	10.9	-10.2	15.8	37
30	R	Little Bear	22	26	45.40	111.28	2154	2.1	14.4	-8.9	40.5	43.4
31	R	Picket Pin	4	1	45.44	110.05	2892	-1.8	9.9	-11	20.6	24.9
32	R	Hellroaring II	20	24	45.04	109.45	2892	-1.4	10.3	-10.4	21.7	36.5
33	R	Sawtel Peak	26	18	44.54	111.41	2400	-0.1	12.9	-11.9	25.9	45.8
34	R	Vinegar Hill	14	26	44.72	118.57	2338	0.5	11.4	-8.6	39.8	54.1
35	S	Mt. Hood	20	22	45.39	121.66	1969	1.7	10.8	-4.9	48.4	15
36	S	Newberry Crater	30	13	43.72	121.23	2100	2.9	12.9	-4.7	45.5	58.1
37	S	Paulina Peak	18	11	43.69	121.25	2250	2	11.9	-5.3	42.3	59.6
38	S	Batchelor Butte	16	1	43.26	122.68	2200	1.9	10.7	-3.9	44.1	40
39	S	Tipsoo Peak	—	13	43.22	122.04	2462	0.7	10	-5.4	34.4	35.3
40	S	Moon Mtn.	6	4	43.20	122.65	2201	1.9	10.8	-3.9	43.9	45.2
41	S	Pelican Butte	40	17	42.51	122.15	2462	1.1	10.4	-5	35.2	50.6
42	S	Ball Mtn.	22	15	41.80	122.16	2363	2.2	11.4	-4.4	39.1	107.1
43	S	Goosenest Summit	6	6	41.72	122.23	2506	1.5	10.6	-4.6	35.3	99.3
44	S	Drakes Peak	26	16	42.30	120.15	2462	2.5	13.1	-5.3	48.1	80.3
45	S	Crane Mountain	19	22	42.07	120.24	2538	2.2	12.7	-5.6	46.3	72.4
46	S	Mt. Rose	11	—	39.30	119.90	2754	2.4	12.6	-4.8	61	88.4
47	S	Stevens Peak	7	—	38.70	119.98	2923	1.6	11	-5.2	46.6	61.6
48	S	Ebbetts Pass	2	—	38.50	119.80	2769	2.5	12	-4.4	46.9	72

Notes: Region: N = northern, R = Rocky Mountain, S = southern; Lat. = latitude, Long. = longitude, Elev. = elevation, See Table 3 for abbreviations and explanation of variables.

means were used to test for differences between treatments for survival percentage using the above model with only the temperature and population effects, and their interaction.

To test differences among populations within each soil temperature, PROC MIXED was used with the REML variance component estimator and the following model:

$$y_{ijklm} = \mu + r_i + b(r)_{ij} + p_k + rp_{ik} + f(p)_{kl} + e_{ijklm}, \tag{Eq. 2}$$

where terms for each effect are the same as in Eq. 1 without the effect of soil temperature. All terms were considered random except for population, which was fixed. To obtain estimates of variance components, the analysis was repeated with all effects considered random.

Genetic differentiation among populations was estimated for all quantitative traits by calculation of Q_{ST} (Spitze, 1993): $Q_{ST} = \sigma^2_b / (\sigma^2_b + 2\sigma^2_w)$, where σ^2_b is the among-population variance and σ^2_w is the within-population additive

genetic variance. In this study the variance component for population (σ^2_p) was used as the among-population variance, and three times the variance component for family within-population ($3\sigma^2_{f(p)}$) was used as the within-population variance. The within-population genetic variation was approximated as three times the family variance instead of four as is used for true half-sibs, because open-pollinated progeny of whitebark pine are more closely related than half-sibs due to moderate inbreeding and correlated paternity (Squillace, 1974; Krakowski et al., 2003; Bower and Aitken, 2007). Values of Q_{ST} were compared to all published estimates for whitebark pine for genetic markers (F_{ST} or G_{ST}).

Climatic variables used in the analyses were mean annual temperature, mean temperature of the coldest month, mean temperature of the warmest month, mean annual precipitation, mean summer precipitation, annual heat:moisture index, summer heat:moisture index, and frost-free period. Climatic variables for populations north of 48°N were obtained from PRISM climatic data corrected for local elevation using the Climate BC model described by Wang et al. (2006a). For populations south of 48°N, climatic data were



Fig. 1. Distribution of *Pinus albicaulis* and locations of populations tested in common-garden experiment. Dashed lines separate the southern, Rocky Mountain and northern regions.

obtained from a model using the thin plate splines of Hutchinson (2000) as illustrated for North America by McKinney et al. (2001). Clines in quantitative traits can be obscured when there are correlations among traits or if geographical structure is complex. In these cases, canonical correlation analysis is more efficient than regressing each trait on environmental variables separately (Westfall, 1992). Several of the seedling phenotypic traits and climatic or geographic variables were strongly intercorrelated (Table 4), so canonical correlation analysis was used to examine the relationships among these variables. The cold

treatment had fewer replications, lack of cold injury testing, and the absence of a few key populations at the northern and southern ends of the range compared to the ambient treatment, thus only data from the ambient treatment were used in the canonical correlation analysis. Climatic data and least-squares population means for each seedling phenotypic trait demonstrating significant ($P \leq 0.05$) population differentiation were included in this analysis. Canonical redundancy analysis was used to determine the proportion of variation in phenotypic traits accounted for by canonical correlations with the climatic or geographic data sets. To assess potential differences in relationships between seedling phenotypic traits and climatic variables between the two soil temperature treatments, canonical correlation analysis was repeated for the two treatments separately using only the populations common to both.

To develop predictive equations for the construction of seed transfer guidelines, values of significant canonical variables for the seedling phenotypic traits were regressed on the standardized climatic variable with the highest loading for that canonical variable. The slope of this regression estimates the rate of change in the phenotypic canonical variable relative to the selected climatic variable. Rates of differentiation along climatic gradients were interpreted relative to the least significant difference among populations at the 20% level (least significant difference: LSD 0.2). This conservatively reduces type II error (accepting no differences among populations when differences actually exist) and minimizes maladaptation risk accordingly (Rehfeldt, 1991). Values of LSD for the phenotypic canonical variables were obtained from a Duncan's multiple range test in PROC GLM using the model for testing variation among populations described. The floating seed transfer model developed by Rehfeldt (1991, 1994) was used to determine seed transfer guidelines for restoration programs of whitebark pine. The maximum recommended environmental transfer distance between seed collection population and planting site was calculated as the difference in the standardized climate variable associated with the LSD ($P = 0.20$) value of the phenotypic canonical variable multiplied by the standard deviation of the climate variable. Univariate regressions of climate variables on latitude, longitude, and elevation were used to determine the geographic distances associated with the rates of differentiation in climate variables to make simple seed transfer recommendations.

RESULTS

Soil temperature effects—Height increment and survival were significantly greater, on average, in the cold treatment than in the ambient treatment (least squares mean = 6.7 and 8.9 mm, $P = 0.04$ for height increment and 66.9 and 82.3%, $P < 0.001$ for survival, in the ambient and cold treatment respectively). Means for biomass traits were also greater in the cold treatment, and the temperature treatment difference greater for root mass than shoot mass, although the difference between treatments for these traits was not significant. The date of needle flush did not differ significantly between treatments. Population-by-treatment interaction was not significant for any of the traits. The foliage of seedlings in the cold temperature treatments generally appeared darker green and healthier than those in the ambient treatment. No treatment-specific geographic trends were evident, and separate canonical correlation analyses of individual treatments yielded the same results.

Geographic patterns across the species range—In general, seedlings from populations originating from colder climates had less overall growth, earlier needle flush in spring, and less cold injury in fall than seedlings originating from milder climates when grown in the common garden. Populations differed significantly in the ambient soil temperature treatment for all variables except root:shoot ratio and spring cold injury (Table 5). Despite a lack of significant differences among populations in the ANOVA, root:shoot ratio differed significantly among populations in a Duncan's multiple range test.

Growth-related traits generally had low levels of population differentiation ($0 \leq Q_{ST} \leq 0.14$), while the cold-adaptation related traits (date of needle flush and fall cold injury) showed

TABLE 3. Description of (A) quantitative and (B) climatic variables.

A) Quantitative trait	Abbreviation	Unit
3rd year height increment	HTINC	millimeters
Total dry biomass	TDM	grams
Root dry biomass	RM	grams
Shoot dry biomass	SM	grams
Root:shoot ratio	RSR	unitless
2003 Date of needle flush	FL03	days from Jan. 1
2004 Date of needle flush	FL04	days from Jan. 1
Fall cold injury	FCI	index of injury (%)
Spring cold injury	SCI	index of injury (%)
B) Climatic variable		
Mean annual temperature	MAT	°C
Mean temperature, warmest month	MTWM	°C
Mean temperature, coldest month	MTCM	°C
Mean annual precipitation	MAP	millimeters
Mean summer precipitation	MSP	millimeters
Annual heat: moisture index	AH:M	$[(MAT + 10)/(MAP/1000)]$
Summer heat: moisture index	SH:M	$[(MWT/(MSP/1000)]$
Frost-free period	FFP	days

TABLE 4. Correlations among population means for quantitative, climatic, and geographic variables. See Table 3 for explanation of variables.

A) Northern region												
Variable	HTINC ^a	TDM ^a	RM ^a	SM ^a	FL03	FL04	FCI	SCI	Survival	Lat.	Long.	Elev.
MAT	-0.15	-0.40	-0.49	-0.31	-0.06	-0.50	-0.16	0.31	0.26	-0.04	0.07	-0.42
MTWM	0.03	-0.16	-0.26	-0.07	0.14	-0.38	-0.21	0.30	0.38	-0.07	0.19	-0.27
MTCM	-0.37	-0.34	-0.48	-0.21	-0.25	-0.04	0.20	0.45	0.13	-0.59*	0.52	0.12
MAP	-0.18	-0.37	-0.41	-0.32	0.02	-0.05	0.35	0.37	-0.03	0.03	-0.19	-0.30
MSP	-0.15	-0.29	-0.33	-0.24	0.20	0.03	0.20	0.50	0.02	-0.09	0.02	-0.14
AH:M	0.33	0.45	0.47	0.43	-0.09	-0.22	-0.55	-0.24	0.17	-0.10	0.31	0.26
SH:M	0.25	0.33	0.31	0.32	-0.17	-0.20	-0.28	-0.41	0.25	-0.04	0.16	0.10
FFP	-0.23	-0.48	-0.52	-0.42	-0.57*	-0.62*	-0.01	-0.42	0.05	0.25	-0.34	-0.63
Lat.	0.41	-0.01	0.14	-0.11	0.10	-0.63*	-0.42	-0.24	-0.08			
Long.	-0.24	0.19	0.04	0.29	-0.07	0.55*	0.19	0.29	0.14	-0.95*		
Elev.	-0.12	0.39	0.30	0.44	0.01	0.76*	0.21	0.30	-0.04	-0.81*	0.86*	
B) Rocky Mountain region												
Variable	HTINC ^a	TDM ^a	RM ^a	SM ^a	FL03	FL04	FCI	SCI	Survival	Lat.	Long.	Elev.
MAT	0.61*	0.60*	0.53*	0.63*	0.04	0.06	0.48	0.53*	0.24	0.20	-0.33	-0.85*
MTWM	0.51*	0.58*	0.54*	0.59*	0.08	-0.14	0.13	0.40	0.13	-0.18	0.11	-0.53*
MTCM	0.44	0.51*	0.46*	0.51*	-0.12	0.23	0.69	0.55*	0.17	0.29	-0.45	-0.71*
MAP	0.21	0.08	0.03	0.11	-0.21	0.01	0.48	0.24	0.18	0.54*	-0.43	-0.53*
MSP	-0.15	-0.15	-0.15	-0.14	-0.43	-0.19	-0.12	-0.11	0.03	0.35	0.22	0.00
AH:M	-0.14	0.00	-0.02	0.03	0.06	0.05	-0.45	-0.12	-0.09	-0.49*	0.40	0.39
SH:M	-0.11	0.08	0.02	0.12	0.34	0.36	0.00	0.17	-0.17	-0.52*	0.08	0.09
FFP	0.41	0.15	0.06	0.22	-0.13	0.00	0.47	0.30	0.33	0.70*	-0.60*	-0.77*
Lat.	0.39	0.01	-0.06	0.07	-0.07	-0.09	0.07	-0.11	0.47*			
Long.	-0.32	-0.07	0.02	-0.12	-0.14	-0.27	-0.66*	-0.27	-0.22	-0.51*		
Elev.	-0.68*	-0.44	-0.34	-0.49*	-0.08	-0.03	-0.50*	-0.39	-0.38	-0.61*	0.66*	
C) Southern region												
Variable	HTINC ^a	TDM ^a	RM ^a	SM ^a	FL03	FL04	FCI	SCI	Survival	Lat.	Long.	Elev.
MAT	0.12	0.24	0.22	0.25	0.05	-0.14	0.22	0.35	-0.32	-0.12	0.47*	-0.03
MTWM	-0.16	0.00	0.00	0.00	0.18	-0.02	0.13	0.51	-0.43	-0.16	0.70*	0.15
MTCM	0.43	0.43	0.42	0.43	-0.26	-0.13	0.17	-0.35	0.24	0.05	-0.60*	-0.28
MAP	0.40	0.20	0.22	0.19	-0.47	-0.20	0.03	-0.36	0.51	0.37	-0.68*	-0.43
MSP	0.46	0.24	0.27	0.22	-0.50	-0.21	-0.05	-0.29	0.55*	0.55*	-0.67	-0.57*
AH:M	-0.17	-0.08	-0.06	-0.11	0.63*	0.55*	-0.07	0.11	-0.43	0.02	0.03	-0.01
SH:M	-0.47	-0.21	-0.23	-0.21	0.59*	0.51	0.00	-0.10	-0.61*	-0.55*	0.26	0.54
FFP	-0.23	-0.15	-0.19	-0.13	-0.08	-0.21	0.38	0.34	-0.25	-0.31	0.65*	0.24
Lat.	0.62*	0.38	0.46	0.31	-0.25	-0.06	-0.23	0.19	0.56*			
Long.	-0.49	-0.31	-0.37	-0.27	0.11	-0.16	0.10	0.26	-0.50	-0.64*		
Elev.	-0.76*	-0.52	-0.58*	-0.47	0.31	0.16	0.10	-0.15	-0.60*	-0.96*	0.67*	

^a Natural log transformed* Significant at $\alpha = 0.05$

moderate to strong differentiation among populations regardless of treatment ($0.36 \leq Q_{ST} \leq 0.65$). A comparison of Q_{ST} values with previously published values of F_{ST} for whitebark pine (Table 1) shows that the phenotypic traits with the weakest differentiation are similar to the highest estimates of differentiation in presumably neutral molecular markers from rangewide studies (Jorgensen and Hamrick, 1997; Richardson et al., 2002b), and the quantitative traits with the strongest differentiation have substantially higher Q_{ST} estimates.

In the canonical correlation analysis of population means for seedling phenotypic traits and climatic variables for population origins, the first canonical correlation was significantly different from zero ($P < 0.0001$) and explained 72% of the variance in the data. The first pair of canonical variables summarizes relationships between cold-related phenotypic traits and mean temperature of the coldest month (Table 6). Mean temperature of the coldest month explained a substantial proportion of the variance in the first phenotypic canonical score ($r^2 = 0.79$, $P < 0.0001$, Fig. 2). The second canonical correlation was also sig-

nificant ($P = 0.006$) and accounted for an additional 15% of the variation. The second pair of variables demonstrates the positive relationship between the length of the frost-free period and growth, both height and biomass (Table 6). The regression of the second phenotypic canonical score on frost-free period was also significant ($P = 0.001$) but weak ($r^2 = 0.24$). Canonical redundancy analysis showed that the first two climatic canonical variables account for 24 and 17% (41% total) of the variation in population phenotypic trait means, indicating substantial genetic structure along climatic gradients.

Regional patterns of variation—When populations were analyzed separately by region, some broad-scale geographic differences in patterns of population differentiation emerged (Table 7). In the ambient soil temperature treatment, in the northern region, significant differences were detected among populations for all three biomass traits. In the Rocky Mountain region, only date of needle flush in 2004 varied significantly among populations, while in the southern region, only date of

TABLE 5. Significance level of population and family within-population effect in ANOVA, over all populations and by region, in two soil temperature treatments.

Variable ^a	Population <i>F</i>	Family in-population <i>Z</i> [†]	Population <i>F</i> by region		
			Northern	Rocky Mtn.	Southern
A-HTINC ^b	1.84**	1.90*	1.59	1.71	1.66
A-TDM ^b	1.69**	2.63**	2.23*	1.20	1.73
A-RM ^b	1.65*	2.12*	2.07*	1.17	1.57
A-SM ^b	1.73**	2.72**	2.47*	1.21	1.78
A-RSR ^b	1.09	1.02	1.34	0.82	1.05
A-FL03	6.51**	2.52**	0.76	0.98	3.25*
A-FL04	5.49**	1.93*	1.70	1.83*	2.12*
A-FCI	2.59**	1.02	1.85	1.17	2.04
A-SCI	1.11	1.25	0.40	0.91	1.76
C-HTINC ^b	1.57*	1.73*	3.52*	2.55**	0.45
C-TDM ^b	1.24	2.06*	1.28	2.56**	0.78
C-RM ^b	1.22	1.86*	1.21	2.31**	0.73
C-SM ^b	1.23	2.14*	1.15	2.30**	0.85
C-RSR ^b	1.09	1.50*	0.30	0.99	1.82
C-FL03	3.29**	1.23	0.47	2.30**	2.01
C-FL04	6.36**	0.48	1.99	3.89**	3.31**

^a A = ambient soil temperature treatment, C = cold soil temperature treatment, see Table 3 for explanation of variables

^b Natural log transformed

* Significant at $\alpha = 0.05$

** Significant at $\alpha = 0.01$

[†] Wald test of covariance parameter = estimate/approximate standard error (SAS Institute, 1999)

needle flush (both years) varied significantly among populations. In the cold treatment, height increment differed significantly among populations in the northern region; all traits except root:shoot ratio differed in the Rocky Mountain region; and date of needle flush differed among populations in the southern region. Estimates of population differentiation (Q_{ST}) were lower, on average, within regions than those for all populations pooled across region (Table 7). In the ambient treatment, the northern region had higher estimates of Q_{ST} , on average, than other regions, while in the cold treatment, estimates of population differentiation were highest for the Rocky Mountain region.

Correlations among quantitative traits and climatic variables also varied by region (Table 4). In the northern region, only the date of needle flush had clinal variation that was positively associated with frost-free period. In the Rocky Mountain region, growth traits (height growth and biomass) and spring cold injury were positively associated with temperature variables. In the southern region, survival was correlated positively with summer precipitation and negatively with summer aridity index, and date of needle flush was positively correlated with aridity.

Scores for the first pair of canonical variables from the southern (Oregon and California) populations were clearly separated from the Rocky Mountain and Canadian populations (Fig. 3). The southern populations had a large influence on the relationship between the first phenotypic canonical score and mean temperature of the coldest month (Fig. 2). Regressions conducted within each region separately revealed a significant relationship between the first phenotypic canonical score and mean temperature of the coldest month in both the northern region ($r^2 = 0.41$, $P = 0.05$) and the Rocky Mountain region ($r^2 = 0.32$, $P = 0.01$), but not in the southern region ($r^2 = 0.08$, $P = 0.38$). The relationship between the second phenotypic canonical score (largely representing growth traits) and frost-free period was only significant in the Rocky Mountain region ($P = 0.003$).

Limits to seed transfer—The interval in mean temperature of the coldest month associated with a significant difference in the first quantitative canonical variable (which largely reflects date of needle flush and fall cold injury) over all regions was estimated as 1.1°C, which translates to a geographic distance of approximately 2.8° latitude or 300 kilometers ($r^2 = 0.49$,

TABLE 6. Correlations between quantitative canonical variables (Quant1 and Quant2) and the quantitative variables, and between climate canonical variables (Clim1 and Clim2) and both climate and quantitative variables.

Variable ^a	Quant1	Quant2	Variable	Clim1	Clim2	Variable	Clim1	Clim2
HTINC ^b	0.07	0.84	MAT	0.75	0.42	HTINC	0.07	0.66
TDM ^b	-0.05	0.58	MTWM	0.22	0.07	TDM	-0.05	0.46
RM ^b	-0.13	0.55	MTCM	0.95	0.27	RM	-0.12	0.43
SM ^b	-0.01	0.59	MAP	0.21	0.62	SM	-0.01	0.46
FL03	0.91	-0.27	MSP	-0.25	0.24	FL03	0.85	-0.21
FL04	0.90	-0.31	AH_M	0.20	-0.31	FL04	0.85	-0.24
FCI	0.66	0.28	SH_M	0.74	-0.35	FCI	0.62	0.22
			FFP	0.14	0.61			

^a See Table 3 for explanation of variables.

^b Natural log transformed

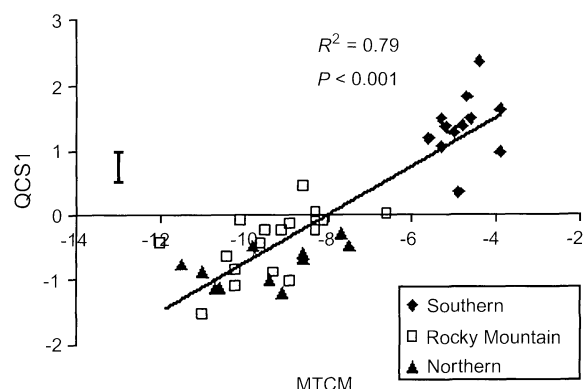


Fig. 2. Regression of first quantitative canonical score (QS1) on standardized mean temperature of the coldest month (MTCM) for 41 populations of *Pinus albicaulis* in three geographic regions. Y-axis scale is standard deviation, and bracket indicates value of LSD 0.20.

$P < 0.0001$ for regression of mean temperature of the coldest month on latitude). The difference for the northern region was 1.9°C and for the Rocky Mountain region was 1.0°C . Mean temperature of the coldest month was most closely associated with latitude in the northern region and with elevation in the Rocky Mountain region (Table 4). The interval in frost-free period associated with the second canonical variable (which primarily comprises growth traits) was 15 d over all regions, which translated to a difference of 1010 m in elevation or 12.2° longitude ($r^2 = 0.23$, $P = 0.001$ for regression of frost-free period on elevation) and 27.5 d in the Rocky Mountain region.

DISCUSSION

Effects of common-garden environments—Common-garden experiments are often replicated in different environments within a species' range to detect genotype-by-environment interactions as well as population differentiation. This experiment was outside of the natural range of whitebark pine; therefore results may have differed from what would have been observed in the natural habitat of this species. A limited supply of avail-

able seed prevented the replication of this experiment in different environments, so the two temperature treatments were intended to assess potential levels of genotype-by-environment interaction. The common-garden environment (at Vancouver, British Columbia; elevation ~ 100 m, MAT = 10°C , MTWM = 17.3°C , MTCM = 3.2°C) is considerably warmer than whitebark pine's native habitat, where frosts can occur during any month of the year (Arno and Hoff, 1989) (Table 2). Although milder air temperatures and warmer soil would enhance growth for most tree species, for whitebark pine, the ambient soil temperature appeared more stressful than the cold soil treatment, even with the soil kept moist. The darker color and superior health of the seedlings in the cold treatment indicated that higher soil temperature was a stress that appeared to be cumulative over the two growing seasons. However, it appears that all populations suffered equally in the warm environment because there was no evidence of genotype-by-environment interaction between soil temperature treatments for any of the traits assessed. Although this experiment was outside of the natural range of the species, phenotypic differences in a common garden among populations demonstrate genetic differences and provide a better basis for estimating limits to seed transfer and likelihood of maladaptation than do estimates from molecular marker studies.

Genetic variation and population differentiation—We observed significant differences among population means in most quantitative traits (Table 5), similar to many other widespread North American conifers (Morgenstern, 1996). In the subalpine environments where whitebark pine grows, traits affecting tolerance of abiotic stresses most likely play a larger role in determining fitness than do growth traits related to competitive ability.

The average level of population differentiation for quantitative traits studied (Q_{ST}) was within the range of previous estimates for other conifers (Merila and Crnokrak, 2001; McKay and Latta, 2002; Howe et al., 2003; Savolainen et al., 2004; St. Clair et al., 2005; St. Clair, 2006; Mimura and Aitken, 2007). Population differentiation (Q_{ST}) was strongest across all populations for traits related to phenology and cold injury. However, patterns of population differentiation varied among regions, with populations in the northern region differentiated most

TABLE 7. Quantitative trait Q_{ST} values over all populations and by region for two temperature treatments.

Variable ^a	All populations	Northern	Rocky Mtn.	Southern
A-HTINC	0.14	0.17	0.13	0.10
A-TDM	0.07	0.24	0.00	0.07
A-RM	0.08	0.37	0.00	0.05
A-SM	0.07	0.30	0.00	0.08
A-RSR	0.00	0.04	0.00	0.00
A-FL03	0.47	0.03	0.00	0.24
A-FL04	0.47	0.08	0.19	0.15
A-FCI	0.36	1.00	0.05	0.25
A-SCI	0.12	0.00	0.05	0.11
C-HTINC	0.13	1.00	1.00	0.00
C-TDM	0.09	0.05	0.89	0.03
C-RM	0.08	0.04	0.48	0.02
C-SM	0.08	0.04	1.00	0.03
C-RSR	0.01	0.00	0.00	1.00
C-FL03	0.43	0.00	0.41	0.12
C-FL04	0.65	1.00	0.47	0.18

^a See Table 3 for explanation of variables.

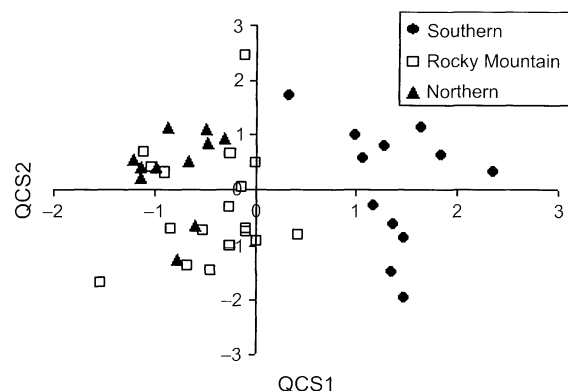


Fig. 3. Scatterplot of first two quantitative canonical scores (QC1 and QC2) based on eight quantitative traits for 41 populations of *Pinus albicaulis*. Axis scales are standardized values. Symbols refer to geographic regions shown in Table 2.

strongly for growth traits (height growth and biomass), populations in the southern region differentiated by cold adaptation traits (date of needle flush and cold injury), while populations in the Rocky Mountain region were differentiated by both height growth and date of needle flush (Table 7). The Q_{ST} values for these traits in these regions were greater than estimates of differentiation in neutral molecular markers (F_{ST} and G_{ST}) for whitebark pine (Table 1). Levels of population differentiation (F_{ST}) previously reported in whitebark pine range from 0.034 to 0.088 and average 0.058, which is slightly higher than most values reported for other stone pine species (*Pinus* subsection *Cembrae*) (Table 1). This value indicates that the vast majority of selectively neutral genetic variation in whitebark pine is among individuals within populations, as for most conifers. Our results show that differentiation is stronger for about half of the quantitative traits we studied than for the upper estimate of differentiation for neutral genetic markers in whitebark pine, indicating a moderate degree of local adaptation. Greater differentiation based quantitative traits than on neutral markers suggests a prominent role of natural selection driving local adaptation of populations for many of these polygenic phenotypic traits (Lynch et al., 1999; Whitlock, 1999).

There is a general concordance of the patterns of variation we detected for seedling phenotypic traits with those reported by both Zavarin et al. (1991) using monoterpenes and Richardson et al. (2002b) using mtDNA. Both of these studies found differentiation among the Rocky Mountain, northern, and southern regions of the species range, which may indicate some historical effects such as isolation, genetic drift, and migration on quantitative genetic structure in addition to the effects of selection for local adaptation to climate. Richardson et al. (2002b) also suggested that genetic evidence supports two Pleistocene refugia for whitebark pine, one in the Yellowstone region and one in the southern Oregon Cascades, with subsequent northward postglacial colonization patterns that have resulted in a secondary contact zone in the southern Washington Cascades. An assessment of quantitative traits across the contact zone suggested by Richardson et al. (2002a, b) might help support the molecular results, but we are unable to test for this pattern due to the lack of representation of Washington Cascade populations in our study.

Environmental gradients associated with phenotypic traits—

Populations differed significantly for nearly all traits, and at a broad geographic scale, those from higher latitude environments with lower winter temperatures flushed earlier in the spring, suffered less cold injury in the fall, and allocated more biomass to shoots in the common-garden environment than those from milder environments. However, clinal variation patterns that corresponded to climatic gradients varied by region (Table 4), indicating local adaptation is driven by selection pressures from different environmental factors in different regions. In the northern region, local adaptation to available growing season length appears to be important because the date of needle flush has a clinal variation associated with frost-free period. In the Rocky Mountain region, annual and seasonal mean temperatures appear to be driving local adaptation, with height growth increasing with temperature at population origins. In the southern region, where survival and date of needle flush were both associated with rainfall patterns, water availability appears to be the factor associated with population differentiation. Regional differences in relationships between phenotypes and source climates have been reported for other

North American conifers, including subalpine fir [*Abies lasiocarpa* (Hook) Nutt.] (Peterson et al., 2002), mountain hemlock [*Tsuga mertensiana* (Bong.) Carr.] (Peterson and Peterson, 2001), and Douglas fir (St. Clair et al., 2005).

Whitebark pine has a high level of cold hardiness compared to other conifers, but significant differences exist among geographic regions (Bower and Aitken, 2006). In the common-garden environment, populations from colder (higher latitude) populations (with lower mean temperature of the coldest month) flushed earlier in the spring and suffered greater spring cold injury. Earlier flushing of these cold-adapted populations may be due to lower chilling requirements in winter, lower heat sum requirements in spring, or both (Howe et al., 2003). In their natural environments, these populations are likely to experience shorter growing seasons than populations further south, so despite a higher risk of spring cold injury, earlier flushing in the spring may be a mechanism to allow trees at these higher latitudes to extend their growing season relative to trees from lower latitudes (Sagnard et al., 2002). In species that can tolerate frost damage well or that have high recovery potential the length of the growing season may be a more important driving force in adaptation than the avoidance of damage (Leinonen and Hanninen, 2002). Worrall (1983) reported differences in both threshold temperature and heat-sum needed for flushing in *Abies amabilis* fir [*Abies amabilis* (Dougl.) Forbes] and subalpine fir and also found that populations from higher elevations flushed earlier than warmer, lower elevation populations in a common garden. A faster response to warming spring temperatures of higher elevation or more northerly sources has also been reported for a number of other conifer species (see references in Campbell and Sugano, 1979; Morgenstern, 1996). For coastal Douglas fir (*P. menziesii* var. *menziesii*), however, where growth may be more limited by drought than cold in some environments, patterns were opposite (Campbell and Sugano, 1979).

Seed transfer guidelines and climate change—While whitebark pine is distributed over a large latitudinal range, the clinal variation observed indicates that trees from a particular population are expected to be optimally adapted for only a portion of the environmental conditions experienced across the species range. We have used the floating seed transfer model developed by Rehfeldt (1991, 1994) to determine seed transfer guidelines for restoration in current climates of whitebark pine. Of the traits we assessed, the date of needle flush gives the strongest signature for local adaptation because it has the highest Q_{ST} estimates (0.43–0.63), thus it was considered first for developing regional estimates of maximum potential seed transfer distances for restoration without substantial risk of maladaptation. In the northern region, it should be possible to move seed from cone collection sites to planting sites that differ by up to 1.9°C in mean temperature of the coldest month and maintain growth phenology suitable for the current local climate with acceptable risk of fall cold injury. In the Rocky Mountain region, the climatic transfer maximum is reduced to 1.0°C in current climates. Restoration ecologists, park managers, and foresters can more easily use seed transfer guidelines based on geographic distances than climatic differences and these differences in mean temperature of the coldest month translate to approximately 4.6° latitude, or 505 km, for the northern region, and 320 m in elevation in the Rocky Mountain region, based on significant correlations between climatic and geographic variables. In the southern region, the lack of correspondence between the first seedling phenotypic canonical variable and mean temperature

of the coldest month suggests that seed can be freely moved within this region. However, in the absence of further data and until populations from the Washington Cascades can be studied and compared with other southern populations, we suggest that transfer between mountain ranges (e.g., Sierra Nevada and Cascades) should be avoided. Height growth and biomass in whitebark pine populations are significantly correlated with the length of the growing season (frost-free period); however, these clines are gentle, and estimates of maximum seed transfer distances based on these traits are also too large to be of practical use in a conservation or restoration program.

The problem with this approach for estimating symmetrical, static seed transfer maxima based on climate is that it assumes local genotypes are optimally adapted to current climates and that these climates will remain constant. The evidence that we are in a period of record rates of warming is mounting (IPCC, 2001). While populations of most temperate and boreal tree species have high levels of genetic variation that could enable adaptation to new environments (Hamrick, 2004), long generation lengths will greatly constrain their ability to adapt to rapid climate change (Burger and Lynch, 1995), and seed dispersal is unlikely to be sufficiently rapid to allow populations to migrate and track climates spatially (Davis and Shaw, 2001). One response to modest climate change may be for trees to migrate within local areas among microsites or aspects. However, whitebark pine inhabits a relatively small range of aspects, slopes, and microsites, so this is not a likely mechanism for maintaining high levels of adaptive diversity. Whitebark pine has reasonably high levels of variation; however, it requires several to many decades to reach sexual maturity, its habitats are discontinuous, largely consisting of high-elevation "islands" separated by lower-elevation valleys, and its migration is dependent on the Clark's nutcracker (Arno and Hoff, 1989). All reduce the likelihood that this species can adapt or migrate sufficiently rapidly to avoid the collapse of many populations. Climate models predict a dramatic decrease in the range of habitat suitable for whitebark pine with increases in temperature and CO₂ (Romme and Turner, 1991; Bartlein et al., 1997; Hamann and Wang, 2006). While increasing temperature may result in new habitat available north of its current range, it is also likely to lead to an upward shift of the timberline and the range of whitebark pine, resulting in a smaller potential area for it to occupy. Many populations currently have negative growth rates due to white pine blister rust infection; fire exclusion and resulting competition from shade-tolerant, fire-intolerant, faster-growing conifer species; and mountain pine beetle (Kendall and Keane, 2001). Interactions among this introduced disease, the current mountain pine beetle epidemic, and climate change could drive populations toward extirpation and the species toward extinction (Hamrick, 2004).

New approaches to developing seed transfer recommendations should balance anticipated future climates with the need to restrict seed movement to environmental distances that can lead to successful seedling establishment under current, albeit transient, conditions. If seed is transferred an excessive distance from warmer to colder climates in anticipation of future warming, cold injury, or mortality may result during establishment; yet if predictions of future climate are ignored, local seed sources that are fit in current local environments may result in restoration plantings of trees that are not adequately competitive with other species or will never achieve reproductive maturity as a result of slow growth rates under the conditions of the decades to come.

One strategy for selecting seed sources that may result in successful establishment of seedlings under current climates, at least some of which can tolerate warming over the next several decades, is to transfer seed unidirectionally to the maximum extent allowable from mild to cold climates on the basis of the estimates from the floating seed transfer model. Most populations of temperate conifers have a reasonably broad temperature tolerance, although populations vary in breadth of reaction norm (e.g., Wang et al., 2006b). To balance the risks of maladaptation in both current and future environments, the challenge is to plant seedlings into environments near their lower-temperature limits to ensure adequate survival and growth, yet have at least some of those trees as adults remain within their temperature tolerances on the warmer side of the reaction norm.

The warming predicted by global circulation models will likely cause phenotypes to shift northward and upward to track conditions to which they are locally adapted (Davis and Shaw, 2001), but climatic differences among regions should maintain clinal variation. The guidelines we propose are established based on a 20% risk of assuming no difference among populations when a difference actually exists. This threshold may be too conservative, given the risks of climate change, and it may become optimal to transfer seed greater distances than estimated and accept a greater risk of type II error (assuming populations are the same when they are different). However, exceeding these distances increases the chance of maladaptation under current conditions and should only be done after weighing this risk against the need for restoration. Mixing seeds from different populations within the acceptable transfer range may also be a reasonable strategy for mitigating uncertain future climates because it may increase the probability that at least some trees survive. Outbreeding depression from the mixing of populations is unlikely to be a problem for whitebark pine; there are no published examples of this phenomenon in conifers that we are aware of, and it is unlikely to evolve in species with high levels of gene flow (e.g., via wind-dispersed pollen).

Successful restoration plantings with preferential movement of seeds from south to north or to higher elevations, both within and exceeding the current species range, could facilitate population migration, particularly if planted genotypes have some resistance to white pine blister rust. Without such human intervention, whitebark pine may continue to decline, and while selection may result in adaptation by favoring survival and reproduction of those local genotypes adapted to new conditions, this may be insufficient demographically to maintain viable populations. A comparison of predictions of the current range of whitebark pine in British Columbia and the current predicted range based on bioclimatic modeling indicates a considerably poorer fit of predicted to actual distribution than is typical of most of the 49 forest tree species in the province (Hamann and Wang, 2006). This lack of fit between actual and current predicted range of whitebark pine may reflect a migrational lag from the dependence on seed dispersal by the Clark's nutcracker and may be indicative of a slow potential rate of migration in response to climate change. This unoccupied potential habitat is also predicted to be one of the few areas that have climates that could support whitebark pine both currently and in seven or eight decades. Facilitated migration via restoration plantings that move blister-rust-resistant genotypes along environmental gradients and into areas of new potential habitat may be the only way that populations of this species can maintain viability. Experimental field plantings will be needed to test this concept operationally.

LITERATURE CITED

- ARNO, S. F., AND R. J. HOFF. 1989. Silvics of whitebark pine (*Pinus albicaulis*), General Technical Report. INT-GTR-253. U.S. Department of Agriculture, Forest Service, Intermountain Research Station, Ogden, Utah, USA.
- BARTLEIN, P. J., C. WHITLOCK, AND S. L. SHAFER. 1997. Future climate in the Yellowstone National Park region and its potential impact on vegetation. *Conservation Biology* 11: 782–792.
- BELOKON, M. M., Y. S. BELOKON, D. V. POLITOV, AND Y. P. ALTUKHOV. 2005. Allozyme polymorphism of Swiss stone pine *Pinus cembra* L. in mountain populations of the Alps and eastern Carpathians. *Russian Journal of Genetics* 41: 1268–1280.
- BOWER, A. D., AND S. N. AITKEN. 2006. Geographic and seasonal variation in cold hardiness of whitebark pine (*Pinus albicaulis* Engelm.). *Canadian Journal of Forest Research* 36: 1842–1850.
- BOWER, A. D., AND S. N. AITKEN. 2007. Mating system and inbreeding depression in whitebark pine (*Pinus albicaulis* Engelm.). *Tree Genetics and Genomes* 3: 379–388. [Online] DOI
- BRUEDERLE, L. P., D. F. TOMBACK, K. K. KELLY, AND R. C. HARDWICK. 1998. Population genetic structure in a bird-dispersed pine, *Pinus albicaulis* (Pinaceae). *Canadian Journal of Botany* 76: 83–90.
- BURGER, R., AND M. LYNCH. 1995. Evolution and extinction in a changing environment: a quantitative genetic analysis. *Evolution; International Journal of Organic Evolution* 49: 151–163.
- BURR, K. E., A. ERAMIAN, AND K. EGGLESTON. 2001. Growing whitebark pine seedlings for restoration. In D. F. Tomback, S. F. Arno, and R. E. Keane [eds.], *Whitebark pine communities: Ecology and restoration*, 325–345. Island Press, Washington, D.C., USA.
- CAMPBELL, R. K. 1979. Genecology of Douglas-fir in a watershed in the Oregon cascades. *Ecology* 60: 1036–1050.
- CAMPBELL, R. K., AND A. I. SUGANO. 1979. Genecology of bud-burst phenology in Douglas-fir: response to flushing temperature and chilling. *Botanical Gazette (Chicago, Ill.)* 140: 223–231.
- DAVIS, M. B., AND R. G. SHAW. 2001. Range shifts and adaptive responses to Quaternary climate change. *Science* 292: 673–679.
- GERNANDT, D. S., G. GEADA LÓPEZ, S. ORTIZ GARCÍA, AND A. LISTON. 2005. Phylogeny and classification of *Pinus*. *Taxon* 54: 29–42.
- GONCHARENKO, G. G., V. E. PADUTOV, AND A. E. SILIN. 1993a. Allozyme variation in natural populations of Eurasian pines. 1. Population structure, genetic variation, and differentiation in *Pinus pumila* (Pall) Regel from Chukotsk and Sakhalin. *Silvae Genetica* 42: 237–246.
- GONCHARENKO, G. G., V. E. PADUTOV, AND A. E. SILIN. 1993b. Allozyme variation in natural populations of Eurasian pines. 2. Genetic variation, diversity, differentiation, and gene flow in *Pinus sibirica* DuRoi in some lowland and mountain populations. *Silvae Genetica* 42: 246–253.
- HAMANN, A., AND T. WANG. 2006. Potential effects of climate change on ecosystem and tree species distribution in British Columbia. *Ecology* 87: 2773–2786.
- HAMRICK, J. L. 2004. Response of forest trees to global environmental changes. *Forest Ecology and Management* 197: 323–335.
- HOFF, R. J., D. E. FERGUSON, G. I. McDONALD, AND R. E. KEANE. 2001. Strategies for managing whitebark pine in the presence of white pine blister rust. In D. F. Tomback, S. F. Arno, and R. E. Keane [eds.], *Whitebark pine communities: Ecology and restoration*, 346–366. Island Press, Washington, D.C., USA.
- 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.
- HUFFORD, K. M., AND S. J. MAZER. 2003. Plant ecotypes: Genetic differentiation in the age of ecological restoration. *Trends in Ecology & Evolution* 18: 147–155.
- HUTCHINS, H. E., AND R. M. LANNER. 1982. The central role of Clark's Nutcracker in the dispersal and establishment of whitebark pine. *Oecologia* 55: 192–201.
- HUTCHINSON, M. F. 2000. ANUSPLIN version 4.1 user's guide. Australian National University, Centre for Resource and Environmental Studies, Canberra, Australia.
- IPCC. 2001. Third Assessment Report of the Intergovernmental Panel on Climate Change IPCC (WG I & II). Cambridge University Press, Cambridge, UK.
- JORGENSEN, S. M., AND J. L. HAMRICK. 1997. Biogeography and population genetics of whitebark pine, *Pinus albicaulis*. *Canadian Journal of Forest Research* 27: 1574–1585.
- KENDALL, K. C., AND R. E. KEANE. 2001. Whitebark pine decline: infection, mortality, and population trends. In D. F. Tomback, S. F. Arno, and R. E. Keane [eds.], *Whitebark pine communities: Ecology and restoration*, 221–242. Island Press, Washington, D.C., USA.
- KRAKOWSKI, J., S. N. AITKEN, AND Y. A. EL-KASSABY. 2003. Inbreeding and conservation genetics in whitebark pine. *Conservation Genetics* 4: 581–593.
- KRUTOVSKII, K. V., D. V. POLITOV, AND Y. P. ALTUKHOV. 1995. Isozyme study of population genetic structure, mating system and phylogenetic relationships of the five stone pine species (subsection *Cembrae*, section *Strobi*, subgenus *Strobus*). In P. Baradat, W. T. Adams, and G. Muller-Starck [eds.], *Population genetics and genetic conservation of forest trees*, 279–304. SPB Academic Publishing, Amsterdam, Netherlands.
- LANNER, R. M. 1982. Adaptations of whitebark pine for seed dispersal by Clark's Nutcracker. *Canadian Journal of Forest Research* 12: 391–402.
- LEINONEN, I., AND H. HANNINEN. 2002. Adaptation of the timing of bud burst of Norway spruce to temperature and boreal climate. *Silva Fennica* 36: 695–701.
- LITTLE, E. L. JR. 1971. Atlas of United States trees, vol. 1, Conifers and important hardwoods. USDA Forest Service Miscellaneous Publication 1146. Washington, D.C., USA.
- LYNCH, M., M. PFENDER, K. SPITZE, N. LEHMAN, J. HICKS, D. ALLEN, L. LATTA, M. OTTENE, F. BOGUE, AND J. COLBOURNE. 1999. The quantitative and molecular genetic architecture of a subdivided species. *Evolution; International Journal of Organic Evolution* 53: 100–110.
- MANN, A. D. 1996. Alpha+: Experimental designs for variety trials and many-treatment experiments. Biometrics and Statistics Scotland, Edinburgh, Scotland.
- MCCOOL, S. F., AND W. A. FREIMUND. 2001. Threatened landscapes and fragile experiences: Conflict in whitebark pine restoration. In D. F. Tomback, S. F. Arno, and R. E. Keane [eds.], *Whitebark pine communities: ecology and restoration*, 263–284. Island Press, Washington, D.C., USA.
- McKAY, J. K., AND R. G. LATTA. 2002. Adaptive population divergence: markers, QTL and traits. *Trends in Ecology & Evolution* 17: 285–291.
- McKAY, J. K., C. E. CHRISTIAN, S. HARRISON, AND K. J. RICE. 2005. "How local is local?" A review of practical and conceptual issues in the genetics of restoration. *Restoration Ecology* 13: 432–440.
- McKINNEY, D. W., M. F. HUTCHINSON, J. L. KESTEVEN, AND L. A. VENIER. 2001. Canada's plant hardiness zones revisited using modern climate interpolation techniques. *Canadian Journal of Plant Science* 81: 129–143.
- MERILA, J., AND P. CRNOKRAK. 2001. Comparison of genetic differentiation at marker loci and quantitative traits. *Journal of Evolutionary Biology* 14: 892–903.
- MIMURA, M., AND S. N. AITKEN. 2007. Adaptive gradients and isolation by distance with postglacial migration in *Picea sitchensis*. *Heredity* 99: 224–232.
- MORGENSTERN, K. E. 1996. Geographic variation in forest trees. UBC Press, Vancouver, British Columbia, Canada.
- PATTERSON, H. D., AND E. R. WILLIAMS. 1976. A new class of resolvable incomplete block designs. *Biometrika* 63: 83–92.
- PETERSON, D. W., AND D. L. PETERSON. 2001. Mountain hemlock growth responds to climatic variability at annual and decadal time scales. *Ecology* 82: 3330–3345.
- PETERSON, D. L., D. W. PETERSON, AND G. J. Ettl. 2002. Growth responses of subalpine fir to climatic variability in the Pacific Northwest. *Canadian Journal of Forest Research* 32: 1503–1517.
- POTENKO, V. V., AND A. V. VELIKOV. 1998. Genetic diversity and differentiation of natural populations of *Pinus koraiensis* (Sieb. et Zucc.) in Russia. *Silvae Genetica* 47: 202–208.

- POTENKO, V. V., AND A. V. VELIKOV. 2001. Allozyme variation and mating system of coastal populations of *Pinus koraiensis* Sieb. et Zucc in Russia. *Silvae Genetica* 50: 117–122.
- PRICE, R. A., A. LISTON, AND S. H. STRAUSS. 1998. Phylogeny and classification of *Pinus*. In D. M. Richardson [ed.], *Ecology and biogeography of Pinus*, 49–91. Cambridge University Press, Cambridge, UK.
- REHFELDT, G. E. 1991. A model of genetic variation for *Pinus ponderosa* in the Inland Northwest (U.S.A.): applications in gene resource management. *Canadian Journal of Forest Research* 21: 1491–1500.
- REHFELDT, G. E. 1994. Adaptation of *Picea engelmannii* populations to the heterogeneous environments of the Intermountain West. *Canadian Journal of Botany* 72: 1197–1208.
- RICHARDSON, B. A., S. J. BRUNSFELD, AND N. B. KLOPFENSTEIN. 2002a. Assessing Clark's nutcracker seed-caching flights using maternally inherited mitochondrial DNA of whitebark pine. *Canadian Journal of Forest Research* 32: 1103–1107.
- RICHARDSON, B. A., S. J. BRUNSFELD, AND N. B. KLOPFENSTEIN. 2002b. DNA from bird-dispersed seed and wind-disseminated pollen provides insights into postglacial colonization and population genetic structure of whitebark pine (*Pinus albicaulis*). *Molecular Ecology* 11: 215–227.
- ROGERS, D. L., C. I. MILLAR, AND R. D. WESTFALL. 1999. Fine-scale genetic structure of whitebark pine (*Pinus albicaulis*): Associations with watershed and growth form. *Evolution; International Journal of Organic Evolution* 53: 74–90.
- ROMME, W. H., AND M. G. TURNER. 1991. Implications of global climate change for biogeographic patterns in the Greater Yellowstone ecosystem. *Conservation Biology* 5: 373–386.
- SAGNARD, F., C. BARBEROT, AND B. FADY. 2002. Structure of genetic diversity in *Abies alba* Mill. from southwestern Alps: multivariate analysis of adaptive and non-adaptive traits for conservation in France. *Forest Ecology and Management* 157: 175–189.
- SAS Institute. 1999. The SAS system for Windows, version 8.0. SAS Institute, Cary, North Carolina, USA.
- SAVOLAINEN, O., F. BOKMA, R. GARCÍA-GIL, P. KOMULAINEN, AND T. REPO. 2004. Genetic variation in cessation of growth and frost hardiness and consequences for adaptation of *Pinus sylvestris* to climatic changes. *Forest Ecology and Management* 197: 79–89.
- SPITZE, K. 1993. Population structure in *Daphnia obtusa*: Quantitative genetic and allozymic variation. *Genetics* 135: 367–374.
- SQUILLACE, A. E. 1974. Average genetic correlations among offspring from open-pollinated forest trees. *Silvae Genetica* 23: 149–156.
- ST. CLAIR, J. B. 2006. Genetic variation in fall cold hardiness in coastal Douglas-fir in western Oregon and Washington. *Canadian Journal of Botany* 84: 1110–1121.
- ST. CLAIR, J. B., N. L. MANDEL, AND K. W. VANCE-BORLAND. 2005. Genecology of Douglas fir in western Oregon and Washington. *Annals of Botany* 96: 1199–1214.
- STUART-SMITH, G. J. 1998. Conservation of whitebark pine in the Canadian Rockies: Blister rust and population genetics. M.Sc. thesis, University of Alberta, Edmonton, Alberta, Canada.
- TANI, N., N. TOMARU, M. ARAKI, AND K. OHBA. 1996. Genetic diversity and differentiation in populations of Japanese stone pine (*Pinus pumila*) in Japan. *Canadian Journal of Forest Research* 26: 1454–1462.
- TOMBACK, D. F. 1978. Foraging strategies of Clark's Nutcracker. *Living Bird* 16: 123–161.
- TOMBACK, D. F. 1982. Dispersal of whitebark pine seeds by Clark's Nutcracker: A mutualism hypothesis. *Journal of Animal Ecology* 51: 451–467.
- TOMBACK, D. F., S. F. ARNO, AND R. E. KEANE. 2001. The compelling case for management intervention. In D. F. Tomback, S. F. Arno, and R. E. Keane [eds.], *Whitebark pine communities: Ecology and restoration*, 3–25. Island Press, Washington, D.C., USA.
- WANG, T., A. HAMANN, D. L. SPITTLEHOUSE, AND S. N. AITKEN. 2006a. Development of scale-free climate data for western Canada for use in resource management. *International Journal of Climatology* 26: 383–397.
- WANG, T. L., A. HAMANN, A. YANCHUK, G. A. O'NEILL, AND S. N. AITKEN. 2006b. Use of response functions in selecting lodgepole pine populations for future climates. *Global Change Biology* 12: 2404–2416.
- WESTFALL, R. D. 1992. Developing seed transfer zones. In L. Fins, S. T. Friedman, and J. V. Brotschol [eds.], *Handbook of quantitative forest genetics*, 313–398. Kluwer, Dordrecht, Netherlands.
- WHITLOCK, M. C. 1999. Neutral additive genetic variance in a metapopulation. *Genetical Research* 74: 215–221.
- WORRALL, J. 1983. Temperature-bud burst relationships in *Amabilis* and subalpine fir provenance tests replicated at different elevations. *Silvae Genetica* 32: 203–209.
- YANDELL, U. G. 1992. An allozyme analysis of whitebark pine (*Pinus albicaulis* Engl.). M.Sc. thesis, University of Nevada, Reno, Nevada, USA.
- ZAVARIN, E., Z. RAFII, L. G. COOL, AND K. SNAJBERK. 1991. Geographic monoterpene variability of *Pinus albicaulis*. *Biochemical Systematics and Ecology* 19: 147–156.