

Geographic patterns of genetic variation and population structure in *Pinus aristata*, Rocky Mountain bristlecone pine

Anna W. Schoettle, Betsy A. Goodrich, Valerie Hipkins, Christopher Richards, and Julie Kray

Abstract: *Pinus aristata* Engelm., Rocky Mountain bristlecone pine, has a narrow core geographic and elevational distribution, occurs in disjunct populations, and is threatened by rapid climate change, white pine blister rust, and bark beetles. Knowledge of genetic diversity and population structure will help guide gene conservation strategies for this species. Sixteen sites across four mountain ranges in the core distribution of *P. aristata* were sampled and genetic diversity was assessed with 21 isozyme loci. Low species and population level genetic diversity ($H_e = 0.070$ and 0.062 , respectively) occurred with moderate among-population differentiation ($F_{ST} = 0.131$). Genetic diversity correlated with longitude, latitude, and elevation and a strong mountain island effect may contribute to substructuring and isolation. Using multiple complementary analyses, sampled trees were assigned to three genetic lineages that varied in diversity and admixture and were associated with different climatic factors. The distribution of genetic diversity and substructuring of *P. aristata* may be an outcome of a combination of restricted gene flow due to geographic and phenological isolation, random processes of genetic drift, life history traits, natural selection, and postglacial migrations. The combination of low genetic diversity, moderate population isolation, and a protracted regeneration dynamic puts populations at risk for extirpation by novel stresses.

Résumé : *Pinus aristata* Engelm., le pin aristé, possède une distribution centrale restreinte, tant géographique qu'altitudinale. L'espèce se retrouve en populations isolées et est menacée par la rapidité des changements climatiques, la rouille vésiculeuse du pin blanc et les scolytes. La connaissance de la diversité génétique et de la structure de populations aidera à orienter les stratégies de conservation des ressources génétiques chez cette espèce. Les auteurs ont échantillonné 16 populations réparties dans les quatre chaînes de montagnes se retrouvant au cœur de la distribution centrale de *P. aristata*. La diversité génétique a été évaluée pour 21 loci d'isoenzymes. Une diversité génétique faible fut remarquée, tant pour l'espèce que dans les populations ($H_e = 0,070$ et $0,062$, respectivement). La différenciation de populations était modérée ($F_{ST} = 0,131$). La diversité génétique était corrélée avec la longitude, la latitude et l'altitude, et un fort effet insulaire dû aux montagnes pourrait contribuer à la sous-structuration et à l'isolement. En recourant à des analyses multiples complémentaires, les arbres échantillonnés pouvaient être assignés à trois lignées génétiques qui variaient quant à leur diversité et leur composition et qui étaient associées à différents facteurs climatiques. La distribution de la diversité génétique et la sous-structuration de *P. aristata* pourraient être la résultante d'une combinaison de facteurs incluant le flux génique restreint en raison de l'isolement géographique ou phénologique, les processus aléatoires découlant de la dérive génétique, les caractéristiques du cycle vital, la sélection naturelle et les migrations postglaciaires. La combinaison de la faible diversité génétique, de l'isolement modéré des populations et d'une dynamique de régénération prolongée résulte augmente les risques que les populations disparaissent à la suite de nouveaux stress.

[Traduit par la Rédaction]

Introduction

Ex situ gene conservation of wild species is of growing interest, as natural populations are increasingly being challenged by introduced pests and pathogens and a changing climate (Guerrant et al. 2004). Programs for *ex situ* seed collections of agronomic species and timber species with breeding programs have been active for years and programs for

wild species for restoration are building (Falk et al. 2006; Walters et al. 2008). Knowledge of the variation and distribution of genetic diversity can guide efficient gene conservation sampling designs (Richards et al. 2007). Genetic information is lacking for many species that provide important ecosystem services yet have not had immediate economic value. Initial tests of geographic variation in genetic diversity provide the

Received 27 May 2011. Accepted 11 July 2011. Published at www.nrcresearchpress.com/cjfr on xx December 2011.

A.W. Schoettle, B.A. Goodrich, and J. Kray. USDA Forest Service, Rocky Mountain Research Station, 240 West Prospect Road, Fort Collins, CO 80526, USA.

V. Hipkins. USDA Forest Service, National Forest Gel Electrophoresis Laboratory, 2480 Carson Road, Placerville, CA 95667, USA.

C. Richards. USDA Agricultural Research Services, National Center for Genetic Resources Preservation, 1111 S. Mason Street, Fort Collins, CO 80523, USA.

Corresponding author: Anna W. Schoettle (e-mail: aschoettle@fs.fed.us).

foundation to design gene conservation strategies and build further studies to test hypotheses of the origins of the observed introgression.

Pinus aristata Englem., Rocky Mountain bristlecone pine, is an example of a species of increasing conservation interest, yet we lack information on its genetic diversity or population structure. *Pinus aristata*, *Pinus longaeva* D.K. Bailey, and *Pinus balfouriana* Balf. comprise the subsection *Balfourianae* Englem. within the subgenus *Strobus*. Together with *Pinus flexilis* E. James and *Pinus albicaulis* Englem., these five species make up the noncommercial high-elevation five-needle white pines of North America and commonly define the forest–alpine ecotones throughout the west. Genetic diversity studies have been published for each of these species, except *P. aristata*, and demonstrate a range of patterns (see Bower et al. 2011). While generalizations have been drawn between genetic diversity and life history traits, the present distribution of genetic diversity for a species is influenced by both evolutionary and natural histories, preventing extrapolation from one species to another (Hamrick and Godt 1996).

The core distribution of *P. aristata* is in central and southern Colorado and extends into northern New Mexico with a disjunct population on the San Francisco Peaks in northern Arizona. The northern limit of the distribution is coincident with the consistent northern reach of the North American Monsoon, suggesting that the species requires mid- to late-summer precipitation (Schoettle 2004). Most *P. aristata* forests are found on isolated mountaintops east of the Continental Divide within an elevational band of 2700–3700 m (Baker 1992). Populations often define the alpine treeline, however the species can also be found in open mixed conifer stands with *Pinus ponderosa* Douglas ex P. Lawson & C. Lawson in central Colorado or with *Pinus edulis* Engelm. in areas of southern Colorado (Ranne et al. 1997).

Some *P. aristata* stands may be among the oldest forests in the Rocky Mountains (Baker 1992). The combination of the pine's ability to survive on harsh sites that also have infrequent stand-replacing disturbances has enabled trees on many sites to reach ages from hundreds to thousands of years old; the oldest living *P. aristata* has been aged at over 2400 years (Brunstein and Yamaguchi 1992). These long-lived trees have the opportunity to contribute to local and long-distance gene flow for thousands of years, which may influence the within-population structure and among-population genetic differentiation. The regeneration dynamic of this early seral species is very protracted with slow recruitment after disturbance on favorable sites and infrequent establishment events on dry exposed sites (Baker 1992; Coop and Schoettle 2009). As a result, *P. aristata* stands are multi-aged (Brown and Schoettle 2008). Once established, maturation of seedlings to seed-bearing trees can take 50–70 years and longer to produce a full seed cone crop. The small winged seeds are wind dispersed but dispersal by Clark's Nutcracker (*Nucifraga columbiana* Wilson) also occurs and may contribute to long-distance colonization events (Torick et al. 1996).

Paleoecology studies suggest that many of the current sub-alpine species of the Southern Rocky Mountains grew on the eastern plains during the last glacial maximum (~18 000 years ago) (Wells and Stewart 1987). Genetic data also support the hypothesis that upon glacial retreat, *P. flexilis*, a species that

frequently coexists with *P. aristata* today, colonized the Southern Rocky Mountains from refugia to the east (Mitton et al. 2000). Glacial refugia for *P. aristata* may also have been along the eastern foothills of the Southern Rocky Mountains with subsequent migration west and north, although this hypothesis is untested. If this is true, however, some populations may be only 10–20 generations from the founding postglacial colonization event and genetic evidence of these or more recent founder populations may remain on the landscape today.

Multiple novel threats currently challenge *P. aristata* including direct and indirect effects of climate change and invasion of a nonnative pathogen. Temperatures have increased 0.5–1 °C throughout the southern Rockies during the last 30 years and high elevations may be warming even more quickly (McWethy et al. 2010). Trends in annual precipitation are less obvious, but there has been a shift toward a greater percentage of precipitation falling as rain rather than snow in the western United States (McWethy et al. 2010). These changes in climatic conditions will likely affect population viability of high-elevation trees like *P. aristata* (Salzer et al. 2009). Population dynamics and current forest structure of many *P. aristata* stands are also at risk from the escalating populations of the mountain pine beetle (*Dendroctonus ponderosae* Hopkins), a native forest pest common throughout the Rocky Mountains that is increasingly prevalent in high-elevation five-needle pine forests due to wintertime warming (Bentz et al. 2010). In addition, *Cronartium ribicola* A. Dietr., the nonnative fungus that causes the lethal disease white pine blister rust, was found for the first time infecting *P. aristata* in the field in 2003 in south-central Colorado (Blodgett and Sullivan 2004). Over the past century, the fungus has spread southward from its point of introduction in the northwest. White pine blister rust is expected to continue to spread through the *P. aristata* distribution, as risk analyses suggest that approximately half of the five-needle pine forests in Colorado annually experience climate conditions that can support *C. ribicola* and the disease on pines and the other half have conducive conditions intermittently (Howell et al. 2006). While *P. aristata* has evolved tolerance to the conditions under which it has historically been exposed, the species' long generation time and low regeneration capacity do not support rapid adaptation to novel stresses. As a result, the combination of these native and novel stresses has increased the urgency to conserve *P. aristata* genetic diversity and manage for sustained pine populations and ecosystem function in these high-elevation ecosystems (Schoettle and Sniezko 2007).

White pine blister rust invasion and climatic warming trends are both relatively recent compared with the generation time of *P. aristata*; thus, a window of opportunity to gather information on this species and its ecosystems under present, noninvaded conditions still exists (Schoettle and Sniezko 2007). The primary objective of this study is to provide the first characterization of patterns of genetic diversity and population structure of *P. aristata*; this information will help guide gene conservation strategies for the species. Because we have no information on the specific evolutionary history of the species, we chose to stratify our sampling by geographic area to be conservative and unbiased in our design. Twenty-one isozyme loci from 615 trees from 16 sites

across four mountain ranges in the core distribution of the species were analyzed for hierarchical genetic structure and evidence of historical lineages. These data are also explored for correlations between the observed genetic pattern with several climate, geographic, and tree age factors.

Methods and materials

Tissue collections

A network of 16 sites across the four major mountain ranges in the core distribution of *P. aristata* in Colorado and New Mexico was designed to encompass the elevational, latitudinal, and climate gradients of the species (Fig. 1; Table 1). Candidate sites within each of four geographic areas were identified from previous work (Ranne et al. 1997), advice from local forest managers, and from our own studies. Final selection of the four populations from each of the four ranges also considered population size and density and site accessibility.

At each site, branch tips were sampled from 34–41 individual trees between 28 June and 12 August 2005 (Table 1). Trees were located at least 33 m apart to avoid sampling related individuals. A unique ID number was assigned to each tree and diameter at breast height (DBH) (1.34 m) and a GPS location were recorded. Three or more terminal sections of 10–15 cm length branches were clipped from each tree and placed in individually labeled freezer bags along with a damp paper towel to prevent tissue desiccation. Bagged samples were stored in soft-sided coolers lined with blue ice in the field and immediately transferred to a larger cooler and kept on blue ice for approximately 12–96 h of transfer time (depending on site), after which they were placed in walk-in coolers in the laboratory. Samples were then packed into styrofoam coolers lined with blue ice and shipped overnight to the USDA Forest Service National Forest Genetic Electrophoresis Laboratory (NFGEL) in Placerville, California.

Tissue preparation and isozyme analysis

A piece of terminal bud tissue, approximately 2 mm² in size, was dissected and submerged in approximately 100 µL of extraction buffer and frozen at –80 °C (USDA Forest Service 2003). Samples were thawed, macerated, and absorbed onto three 3 mm paper wicks on the morning of the electrophoretic run. Extracts were separated on 11% starch gels (Starch-Art) and stained for 21 enzymes resolved on three different buffer systems. A lithium borate electrode buffer (pH 8.3) was used with a Tris–citrate gel buffer (pH 8.3) to resolve LAP-1 and LAP-2, PGI-1 and PGI-2, and PGM; a sodium borate electrode buffer (pH 8.0) was used with a Tris–citrate gel buffer (pH 8.8) to resolve UGPP, TPI, GDH, CAT-1 and CAT-2, and AAT-1 and AAT-2; and a morpholine citrate electrode and gel buffer (pH 8.0) was used to resolve MDH-1, MDH-2, MDH-3, and MDH-4, IDH, 6PGD, MNR, and SKD-1 and SKD-2. For quality control purposes, all samples were scored twice and conflicts resolved. Additionally, 10% of samples were genotyped twice. All loci had a 0% error rate except for the following: CAT-1 = 1.6%, AAT-1 = 1.6%, MDH-2 = 1.6%, PGI-2 = 3.3%, PGM = 3.3%, and MNR = 6.6%.

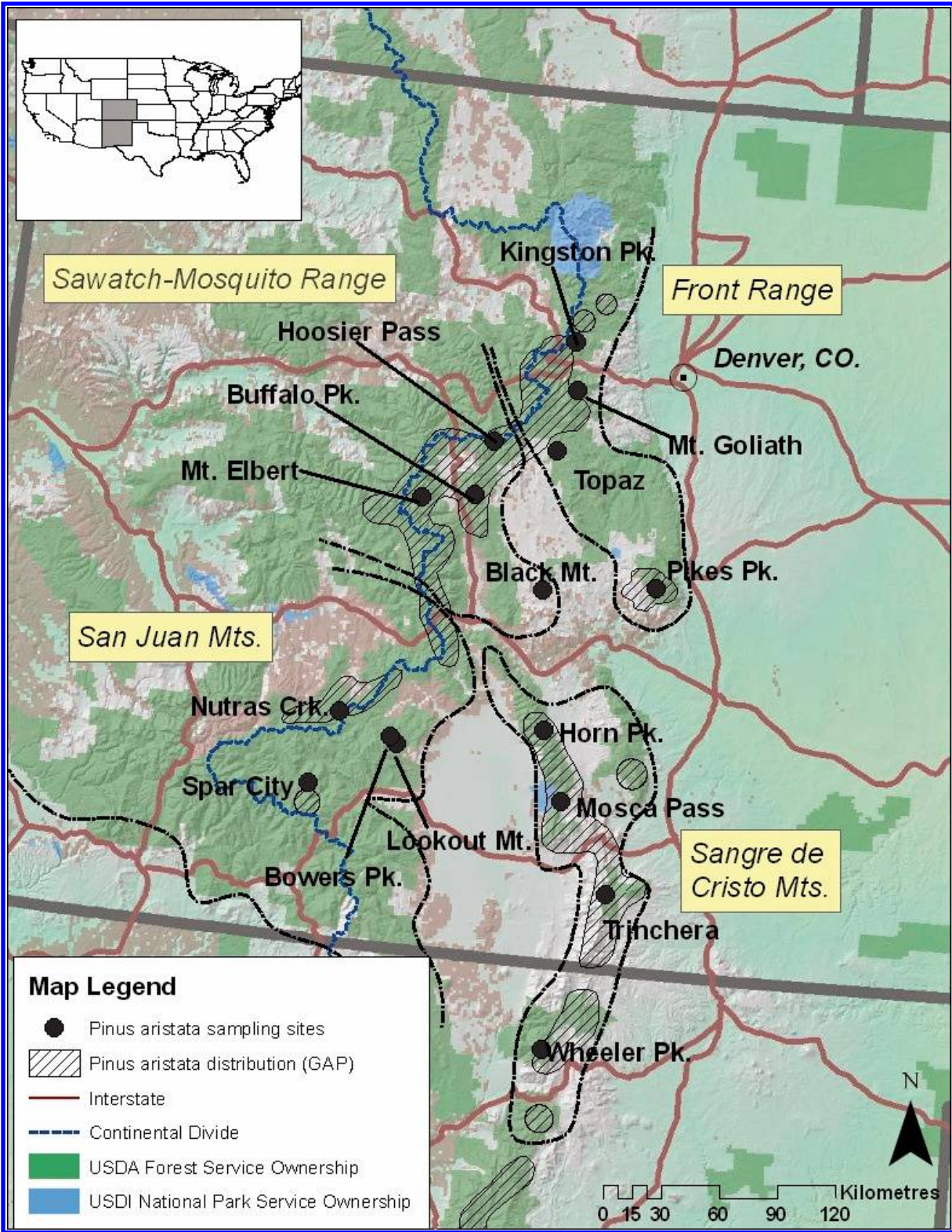
Statistical analyses

Diversity statistics were calculated using Genetic Data Analysis (GDA) software (Lewis and Zaykin 2001) and FSTAT (Goudet 1995). To describe the genetic variation observed in each site, we estimated observed and expected heterozygosity, the fixation index (F_{IS}), and the number of private alleles for each site and mountain range and over all sites. A hierarchical structure of sampling sites within mountain range was used in both analyses to determine diversity statistics by mountain range (Table 1). We also estimated genetic diversity statistics for each locus including percent polymorphism, mean number of alleles, observed and expected heterozygosity, and F_{IS} . Analysis of genetic structure was estimated with both GDA and FSTAT. Nei's genetic distance was estimated for each pair of populations using GenAlEx version 6.3 (Nei 1972; Peakall and Smouse 2006). These genetic distances were then used to construct a consensus phenogram, bootstrapped over 1000 replicates, using the neighbor-joining method employed by PHYLIP (Felsenstein 1993).

Genetic differentiation among *P. aristata* sites was further described using two other statistical methods. Geographic variation was estimated by canonical correlation analysis (CCA) by the methods described in Westfall and Conkle (1992). Specifically, a second-order surface equation of latitude, longitude, and elevation (in metres) was regressed against allelic frequencies. The directions of the geographic differentiation were shown in a contour plot of predicted scores from the statistically significant patterns in the CCA. The Correlation Procedure was used on diversity statistics and geographic locations of sampling sites to determine significant linear relationships (SAS version 9.3).

An alternative approach to estimating genetic structure was used that requires no prior information of sampling location. We used two complementary implementations of a Bayesian clustering algorithm to determine the number of lineages from the genotypic data using the software (i) STRUCTURE with Structure Harvester and (ii) STRUCTURAMA as well as analyses to test for stability of the lineage number. Following recommendations and methods from Evanno et al. (2005) to determine K (lineages), we used the Markov chain Monte Carlo (MCMC) algorithm and the Bayesian framework of STRUCTURE and ran 10 000 MCMC replications after a burn-in length of 10 000 for every possible number of lineages (K) from 1 to 16. Twenty runs were completed for each K to view the variance of the proportions between runs. We utilized the online version of Structure Harvester version 0.56.4 (Evanno et al. 2005; Earl 2009) to define the most likely number of lineages. The magnitude of ΔK has been used as a criterion to infer the most probable number of lineages represented by the samples, especially when sampling is even across populations and large (Evanno et al. 2005). ΔK is calculated as $m(l(K+1) - 2L(K) + L(K-1))/SD[L(K)]$, where m is the mean and SD is the standard deviation (Evanno et al. 2005). Alternatively, population structure was estimated using a Bayesian, MCMC method of Huelsenbeck and Andolfatto (2007) implemented in the software STRUCTURAMA. K was treated as a random variable, with the prior set to 2 to seed the program. A single Markov chain was run for 1×10^6 generations. The first half of the run was treated as the burn-in period, after which the chain was

Fig. 1. Location of 16 *Pinus aristata* sites sampled for genetic variation across four mountain ranges in the core distribution of the species in Colorado and New Mexico (USA).



sampled every 500 generations. A total of 100 independent runs were conducted, each of which provided an estimate of K as the number of lineages with the highest posterior probability. Our reported K was inferred as the mode of the K values estimated by each independent run.

Since individuals are assigned probabilistically for some values of K , individuals may be assigned to different lineages in independent MCMC runs. The consistency of an individual's assignment in these runs has been used as a quantitative measure supporting a particular value of K (Rosenberg et al.

Table 1. Names, sampling dates, locations, mean elevations, and mean diameters of 16 populations (sites) of *Pinus aristata* sampled for genetic analysis in the core distribution across Colorado and New Mexico.

	National Forest	<i>N</i>	Sampling date (month/day/year)	Location (latitude, longitude)	Elevation (m)	DBH (cm)
Front Range						
Topaz	Pike	35	6/28/05	39°20'N, 105°41'W	2883	31.8
Kingston Peak	Roosevelt	35	7/26/05	39°51'N, 105°38'W	3385	21.9
Mt. Goliath	Arapaho	41	7/12/05	39°38'N, 105°36'W	3603	30.5
Pikes Peak	Pike	40	8/02/05	38°45'N, 105°00'W	3233	18.4
Sawatch-Mosquito Range						
Buffalo Peak	Pike	40	7/03/05	39°06'N, 106°09'W	3375	25.0
Hoosier Pass	Pike	35	7/02/05	39°21'N, 106°04'W	3590	23.2
Black Mountain	Pike	40	7/04/05	38°42'N, 105°41'W	3176	29.1
Mt. Elbert	San Isabel	34	7/04/05	39°04'N, 106°28'W	3235	29.6
Sangre de Cristo Range						
Horn Peak	San Isabel	40	8/09/05	38°03'N, 105°35'W	3538	21.1
Mosca Pass	San Isabel	40	8/10/05	37°44'N, 105°27'W	3158	18.7
Trinchera	San Isabel	40	8/11/05	37°20'N, 105°08'W	3284	16.8
Wheeler Peak	Carson	35	8/12/05	36°36'N, 105°25'W	3522	16.3
San Juan Range						
Nutras Creek	Gunnison	40	7/19/05	38°03'N, 106°49'W	3165	23.3
Lookout Mountain	Rio Grande	40	7/20/05	37°55'N, 106°27'W	2900	28.3
Bowers Peak	Rio Grande	40	7/20/05	37°57'N, 106°30'W	3117	41.4
Spar City	Rio Grande	40	7/21/05	37°42'N, 106°57'W	3173	24.8

Note: *N*, number of trees sampled per population; DBH, average diameter at breast height (1.34 m).

2002). *K* values that result in little variation among assignments in repeated runs are considered statistically robust. To estimate *K* using this criterion, we calculated a stability coefficient among 25 MCMC runs for each value of *K* from 1 to 16 (Richards et al. 2009).

Lineage assignments of individual trees from STRUCTURAMA output were then analyzed in STRUCTURE 2.3.3 (Pritchard et al. 2000) to determine the amount of admixture within each lineage. The 615 trees were coded with the STRUCTURAMA lineage assignments defined as “putative population origin” (with no regard to their original population origins) and run through STRUCTURE (with *K* set to 3). We varied both the burn-in length and the MCMC replications from 10 000 to 100 000 and found that it did not affect assignments or the ratio values. Final runs were done using 100 000 for the replication number and burn-in length. For each run, we chose the admixture model under the assumption of correlated allele frequencies between lineages and allowed the degree of admixture alpha to be inferred from the data (Pritchard et al. 2000; Evanno et al. 2005). Allelic richness was calculated for individuals definitively assigned to each STRUCTURAMA lineage in FSTAT 2.9.3.2 (Goudet 1995) using rarefaction techniques for unequal sample sizes. Gene diversity statistics (*H_e*) for each lineage were also calculated in FSTAT.

Latitude, longitude, and elevation for each sampled tree were averaged to create a set of mean coordinates and elevation for each site. Climate variables were derived from monthly climatic surfaces using Hutchinson’s thin-plate splines that fit climate data to geographic surfaces to provide point predictions for numerous temperature and precipitation related variables (Rehfeldt 2006) (<http://forest.moscowfs.wsu.edu/climate/customData/> (Rocky Mountain Research Station, Moscow Forestry Sciences Laboratory)).

Using tree DBH as a potential proxy for tree age, the STRUCTURAMA lineage assignments of the 10 largest trees at each site were compared with the STRUCTURAMA lineage assignments of all trees at the same site to determine if distributions differed. Individual χ^2 tests by site tested the assignments of large trees compared with all trees at any site using the chisq option in Proc Freq (SAS version 9.3). A χ^2 test also compared the proportional assignment of the 160 largest trees with the proportional assignments of all 615 individual trees.

Results

Genetic diversity

Twenty-one loci from 615 sample trees from 16 *P. aristata* sampling sites were used to determine genetic diversity (Tables 2 and 3). The number of alleles per locus ranged from 1 to 4 (Table 3). Expected heterozygosity ranged from 0.0 at five different loci to a high of 0.297 at one of the remaining 16 polymorphic loci (Table 3). Within-population genetic diversity (expected heterozygosity, *H_e*) averaged 0.062 and the species-level *H_e* was 0.070 (Table 2). The *H_e* of *P. aristata* sampling sites ranged approximately sevenfold from 0.016 (Spar City) to 0.113 (Mt. Goliath) (Table 2). Genetic diversity varied slightly within each mountain range, from a low *H_e* of 0.033 in the San Juan Mountains to 0.091 in the Front Range Mountains (Table 2). In contrast, the San Juan Mountain samples had the highest percent polymorphic loci (52.5%), while the Front Range Mountain samples had the lowest (42.9%). An overall deficiency of heterozygosity occurred at all levels of analyses (species, range, and sampling site), as indicated by positive *F* values (Table 2).

Ten sites contained locally common alleles found in less than a quarter of the sites, and all 16 sites contained alleles

Can. J. For. Res. Downloaded from www.nrcresearchpress.com by CSP Staff on 12/19/11
For personal use only.

Table 2. Genetic diversity statistics of *Pinus aristata* by mountain range, sampling sites (populations), and species.

Mountain range and sampling site	<i>N</i>	<i>H</i> _o	<i>H</i> _e	<i>F</i> _{IS}	<i>F</i> _{ST}	<i>F</i> _{ST} 95% CI
Front Range						
Topaz	35.0	0.035	0.044	0.22		
Kingston Peak	34.7	0.062	0.090	0.31		
Mt. Goliath*	39.6	0.049	0.113	0.57		
Pikes Peak*	39.0	0.045	0.061	0.26		
Among sites	144.9	0.048	0.091	0.47	0.129	0.037–0.205
Sawatch-Mosquito Range						
Buffalo Peak	37.6	0.057	0.064	0.11		
Hoosier Pass	33.6	0.038	0.087	0.56		
Black Mountain*	39.9	0.036	0.065	0.46		
Mt. Elbert	34.0	0.031	0.068	0.55		
Among sites	145.1	0.041	0.080	0.49	0.098	0.035–0.177
Sangre de Cristo Range						
Horn Peak*	38.9	0.026	0.029	0.10		
Mosca Pass	39.3	0.032	0.066	0.53		
Trinchera*	37.3	0.048	0.089	0.46		
Wheeler Peak	32.9	0.052	0.086	0.40		
Among sites	148.4	0.039	0.071	0.45	0.109	0.052–0.144
San Juan Range						
Nutras	39.9	0.014	0.026	0.46		
Bowers Peak	39.9	0.026	0.041	0.37		
Lookout Mountain	39.3	0.010	0.042	0.77		
Spar City	39.4	0.010	0.016	0.42		
Among sites	158.6	0.015	0.033	0.55	0.084	0.019–0.132
All ranges						
Among mountain ranges	149.2	0.036	0.069	0.48	0.059	0.038–0.075
Among all sites (population mean)	37.5	0.036	0.062	0.43	0.131	0.069–0.179
Among all individuals (species mean)	597.0	0.035	0.070	0.50		

Note: *N*, sample size; *H*_o, observed heterozygosity (no. of heterozygotes/*N*); *H*_e, expected heterozygosity (1 – sum pi²), where pi is the frequency of the *i*th allele for the population and sum pi² is the sum of the squared population allele frequencies; *F*_{IS}, fixation index ((*H*_e – *H*_o)/*H*_e = 1 – (*H*_o/*H*_e)); *F*_{ST}, population differentiation. An asterisk indicates that the sampling site contained private allele(s).

found in less than half of the sites (data not shown). Five sites contained private alleles: Pikes Peak, Mt. Goliath, Horn Peak, Black Mountain, and Trinchera (Table 2). The relationship between *H*_e and the proportion of alleles local to less than half of the sites was positive and significant (*p* = 0.017) (data not shown), indicating that rare alleles are an important component of genetic diversity in *P. aristata*. However, all of the rare alleles were at very low frequency (less than 0.02) and therefore did not contribute substantially to differentiation.

Population structure

The proportion of genetic variation residing among *P. aristata* sites (populations, *F*_{ST}) was 0.131 (Table 2). Genetic differentiation among mountain ranges was 0.059 in single-level analysis (i.e., individuals within mountain ranges) (Table 2). Population differentiation of individuals within each mountain range spanned from *F*_{ST} = 0.084 (San Juan Range) to *F*_{ST} = 0.129 (Front Range) (Table 2).

Genetic diversity was not distributed randomly across the landscape. A positive relationship occurred between site longitude and *H*_e; those at lower longitudes (farther west) had lower *H*_e than those at higher longitudes (Pearson’s correlation coefficient (*r*) = 0.57, *p* = 0.02) (Fig. 2a). A significant positive correlation also occurred between expected heterozygosity and the elevation of the populations (*r* = 0.62, *p* =

0.01) (Fig. 2b). Simple correlation coefficients between latitude and *H*_e were not significant (Fig. 2c).

A CCA revealed further structure. The first two vectors of the CCA were significant and accounted for 32% of the total variation. The first vector (pattern) was in the form of that for isolation by distance and accounted for approximately 22% of the variation (Fig. 3). A genetically continuous area is visible in a contour plot from the west-central area near the Bowers Peak and Lookout Mt. sites to the north near the Topaz site (Fig. 3). The second significant vector in the CCA was a weak north–south cline that accounted for 10% of the total variation (data not shown).

Population lineages

The number of lineages the individual trees were assigned to was indicated by the ad hoc statistic of Δ*K* based on the rate of change in the log probability of data between successive *K* values (Evanno et al. 2005) and methods to determine stability analyses (Richards et al. 2009). Both methods determined the most likely number of genetic lineages (*K*) to be 3. STRUCTURAMA definitively assigned each individual tree to one of the three inferred lineages and the proportion of individuals assigned to each lineage at each site is reported in Table 4. Overall, 517 individuals were assigned to Lineage 1, 64 individuals to Lineage 2, and 34 individuals to Lineage 3. Individuals in Lineage 1 were more admixed (maximum

Can. J. For. Res. Downloaded from www.nrcresearchpress.com by CSP Staff on 12/19/11
For personal use only.

Table 3. Genetic diversity statistics of *Pinus aristata* by locus.

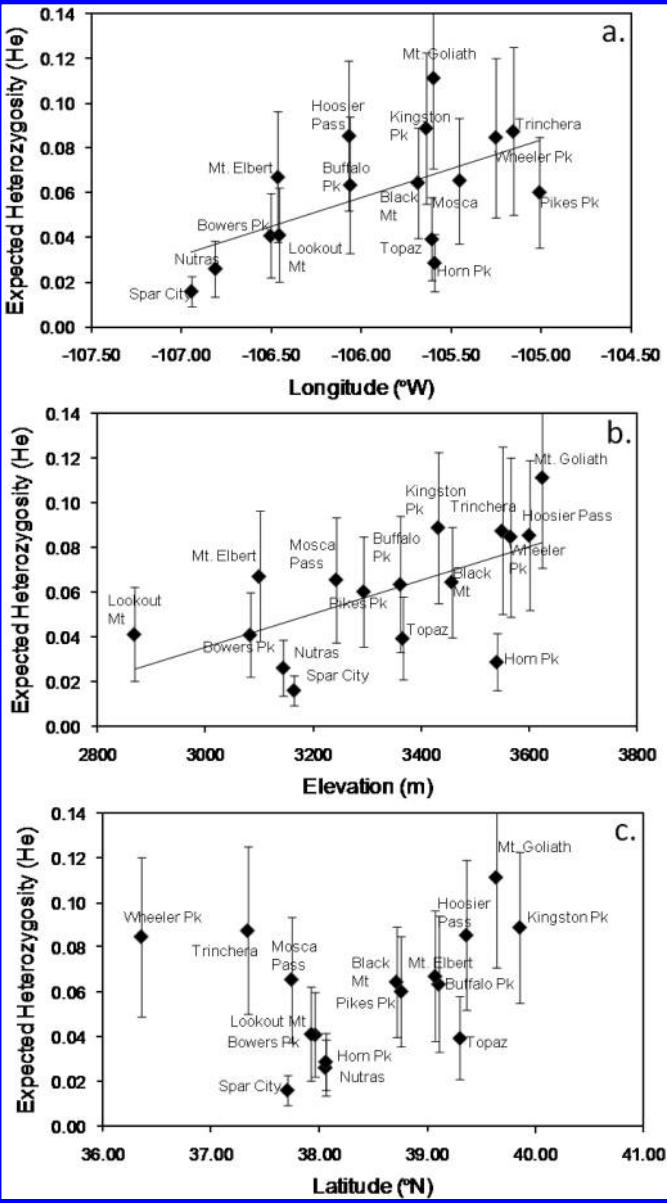
Locus	N	A	H _e	H _o	F _{IS}
PGM	594	3.0	0.268	0.184	0.31
LAP-1	614	2.0	0.006	0.000	1.00
LAP-2	612	2.0	0.002	0.002	0.00
PGI-1	615	1.0	0.000	0.000	0.00
PGI-2	615	4.0	0.150	0.130	0.13
UGPP	615	2.0	0.002	0.002	0.00
TPI	615	1.0	0.000	0.000	0.00
AAT-1	614	4.0	0.297	0.171	0.43
AAT-2	615	1.0	0.000	0.000	0.00
GDH	614	2.0	0.006	0.000	1.00
MDH-1	615	1.0	0.000	0.000	0.00
MDH-2	615	2.0	0.035	0.000	1.00
MDH-3	615	3.0	0.010	0.007	0.33
MDH-4	613	2.0	0.005	0.002	0.67
IDH	615	2.0	0.002	0.002	0.00
SKD-1	614	2.0	0.003	0.003	0.00
SKD-2	615	2.0	0.002	0.002	0.00
6PGD	615	1.0	0.000	0.000	0.00
MNR	615	2.0	0.277	0.224	0.19
CAT-1	443	3.0	0.277	0.011	0.96
CAT-2	438	2.0	0.136	0.000	1.00
All	5974	2.1	0.070	0.035	0.50

Note: N, sample size; A, mean alleles per locus; H_e, expected heterozygosity ($1 - \sum p_i^2$), where p_i is the frequency of the ith allele for the population and $\sum p_i^2$ is the sum of the squared population allele frequencies; H_o, observed heterozygosity (no. of heterozygotes/N); F_{IS}, fixation index ($(H_e - H_o)/H_e = 1 - (H_o/H_e)$).

average membership coefficient within lineage ($Q_{\max}$ = 0.494) compared with individuals in Lineage 2 ($Q_{\max}$ = 0.878) and Lineage 3 ($Q_{\max}$ = 0.891) (Table 4). Lineage 3 had higher allelic richness (1.57) based on rarefaction methods for unequal sample sizes (Petit et al. 1998) than Lineage 1 or 2 (1.28 and 1.42, respectively) (Table 4). Mean H_e was also greatest for Lineage 3 (0.105) (Table 4).

While the majority of all individuals at each sampling site were probabilistically assigned to Lineage 1 (517 out of 615 total individual samples), some sites had more diverse composition than others (i.e., high proportions of individuals in Lineages 2 and 3) (Table 4; Fig. 4). To reflect the within-population diversity in lineage assignments, we defined three site groupings: Group 1 has most of the trees assigned to Lineage 1, Group 2 included those sites with high proportions of individuals assigned to Lineage 2, and Group 3 included those populations with high proportions of individuals assigned to Lineage 3 (see Table 4). Some sites grouped in accordance with the mountain range from which they were sampled, for example, Mosca Pass, Trinchera, and Wheeler Peak all from the Sangre de Cristo Mountains are in Group 3 with high proportions of individuals assigned to Lineage 3 (Table 4; Fig. 4). A large proportion of individuals at Pikes Peak were also assigned to Lineage 3 (Table 4; Fig. 4). Group 2, those sites with a high proportion of individuals assigned to Lineage 2, included Kingston Peak, Mt. Goliath, Hoosier Pass, and Mt. Elbert of the northern Front Range and Sawatch-Mosquito Range and Lookout Mt. of the San Juan Mountains (Table 4; Fig. 4). The other sampling sites in the San Juan Mountain sites, including Bowers Peak,

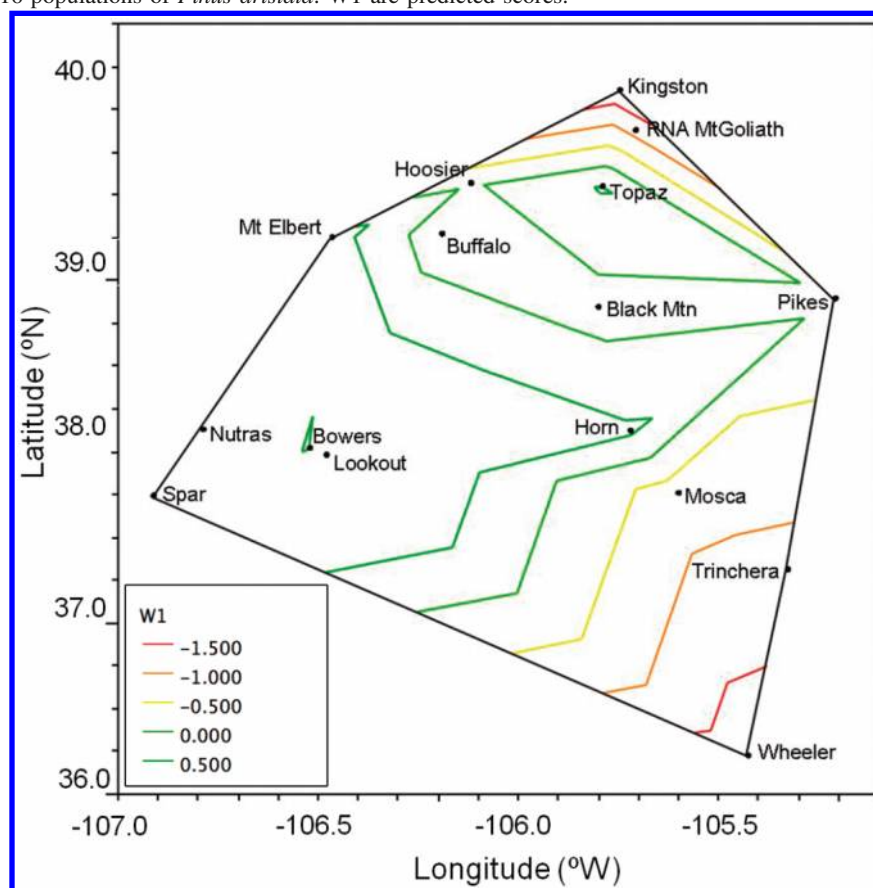
Fig. 2. Relationships between (a) population expected heterozygosity and longitude (Pearson's correlation coefficient (r) = 0.57, p = 0.02), (b) population expected heterozygosity and elevation (r = 0.62, p = 0.01), and (c) population expected heterozygosity and latitude (not significant) of 16 *Pinus aristata* populations sampled in Colorado and New Mexico. Means represent mean diversity statistics and standard error bars represent ± 1.0 SE, both generated by GenAlEx 6.3.



Nutras, and Spar City, were in Group 1 with over 90% of individuals at each site assigned to Lineage 1 (Table 4). Other sites in Group 1 were Topaz, Black Mountain, Buffalo Peak, and Horn Peak in the central area of the *P. aristata* core range (Table 4; Fig. 4).

A consensus neighbor-joining phenogram based on Nei's (1972) genetic distance supported much of the STRUCT-AMA lineage assignments for *P. aristata* (Fig. 5). The south and eastern-most populations that formed Group 3, as defined by the distribution of lineage assignments within each site, also formed a close neighbor grouping in the phenogram

Fig. 3. Contour plot for 16 populations of *Pinus aristata*. W1 are predicted scores.



(Table 4; Fig. 5). Likewise, the northern populations that formed Group 2 in the ancestral analyses also formed a distinct subclade on the phenogram, with the exception of Kingston Peak (Figs. 4 and 5). The phenogram is also consistent with the CCA results in that the area spanning Bowers Peak and Topaz is central to the other outlying populations (Figs. 3 and 5).

Relationships between population structure and site and tree characteristics

Climate varied among the three groupings based on distributions of assignment proportions (Table 4). Some apparent temperature differences occurred between Groups 1, 2, and 3, including warmer mean annual and July temperatures, longer frost-free periods, and a warmer mean temperature in the coldest month at sites in Group 3 (Table 4). Group 3 included more southern sites than the other groups and had both higher mean annual and growing season precipitation (Table 4; Fig. 4). Group 3 generally had the largest deviation from the average climate of all *P. aristata* sites (Table 4).

To explore if lineage differences were associated with different-aged individuals, the genetic diversity signature of older trees was compared with that of all of the sample trees. Using tree diameter as a proxy for tree age, the STRUCTURAMA lineage assignments of the 10 largest trees at each site were compared with the assignments of all trees at the same site. Individual χ^2 tests by site revealed that the assignments of large trees (mean DBH = 37.6 cm, SE = 1.44) compared with all trees at the site (mean DBH =

26.2 cm, SE = 0.63) did not differ for any site (p values for significance by site ranged from 0.24 to 0.95) (site data not shown). A χ^2 test comparing the 160 largest tree assignments with the 615 individual tree assignments also revealed that there were no differences between the lineage assignment distribution of the largest trees only (80.0%, 13.1%, and 6.9% for Lineages 1, 2, and 3, respectively) and the distribution of all of the trees (84.1%, 10.4%, and 5.5% for Lineages 1, 2, and 3, respectively) ($p = 0.471$).

Discussion

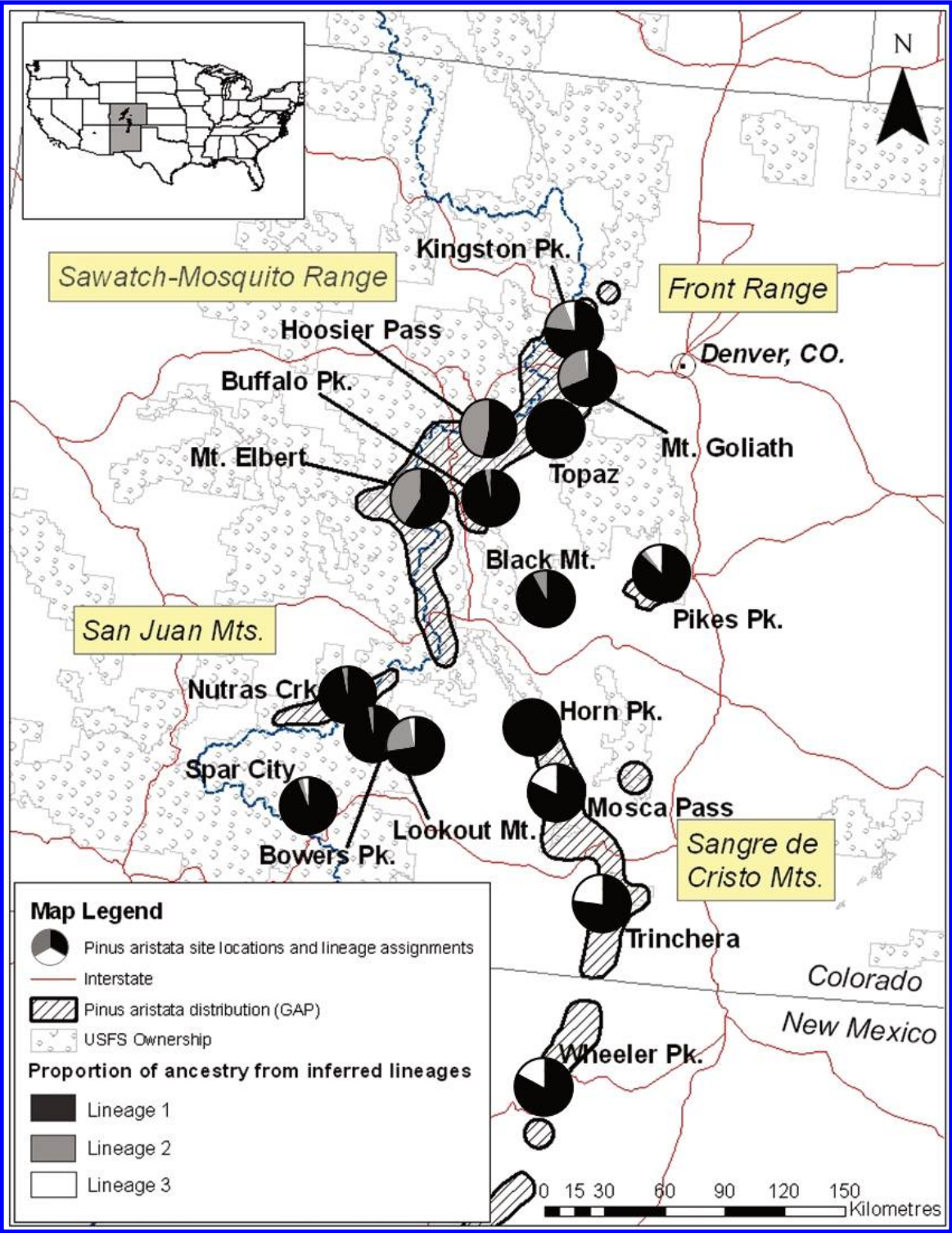
Trees, with their long generation time and high outcrossing rates, typically have more genetic diversity within populations than other life forms and those tree species with broad distributions have greater diversity than species with more narrow ranges (Hamrick and Godt 1990; Hamrick et al. 1992). Consistent with *P. aristata*'s narrow distribution, its genetic diversity at the population level is lower than that of most other long-lived woody perennials ($H_e = 0.062$ versus 0.148, respectively) and more similar to that of other endemic long-lived woody species ($H_e = 0.062$ versus 0.056, respectively) (see tables 1 and 3 in Hamrick et al. 1992). Genetic diversity of *P. aristata* is lower than that of any of the other high-elevation five-needle pines (population- and species-level H_e from isozyme studies range from 0.075 to 0.327; see Bower et al. 2011). The diversity of *P. aristata* was even lower than that of its close relatives in the *Balfourianae* section that also have narrow distributions (*P. balfouriana* $H_e =$

Table 4. Site mean proportion of assignment probability for each individual *Pinus aristata* using STRUCTURAMA (Huelsenbeck and Andolfatto 2007) and mean and standard errors of 30-year (1971–2000) averages for climate variables of interest (<http://forest.moscowsl.wsu.edu/climate/>) averaged over sites within each group (Group 1 = 7 sites, Group 2 = 5 sites, and Group 3 = 4 sites).

Groups and sites	Proportion of trees in STRUCTURAMA assignments			30-year average climate variables by Group					
	Lineage 1	Lineage 2	Lineage 3	Mean annual temperature (C°)	July mean temperature (C°)	Mean annual precipitation (mm)	Growing season precipitation (mm)	Mean temperature in the coldest month (C°)	Frost-free period (days)
Group 1				0.49 (0.34)	11.79 (0.22)	583.29 (48.32)	322.57 (14.51)	−10.09 (0.43)	32.1 (4.85)
Topaz	1.00	0.00	0.00						
Buffalo	0.97	0.03	0.00						
Black Mt.	0.92	0.08	0.00						
Horn Peak	1.00	0.00	0.00						
Spar City	0.94	0.03	0.03						
Nutras	0.97	0.03	0.00						
Bowers Peak	0.97	0.03	0.00						
Group 2				−0.60 (0.74)	11.14 (0.68)	596.20 (43.95)	339.60 (34.56)	−10.88 (0.68)	31.4 (6.10)
Kingston Peak	0.77	0.17	0.06						
Hoosier	0.54	0.46	0.00						
Mt. Elbert	0.59	0.41	0.00						
Mt. Goliath	0.69	0.29	0.02						
Lookout Mt.	0.72	0.25	0.03						
Group 3				1.6 (0.52)	12.48 (0.81)	618.5 (83.99)	394.00 (43.33)	−7.93 (0.37)	42.5 (3.62)
Pikes Peak	0.87	0.03	0.10						
Mosca Pass	0.82	0.00	0.18						
Trinchera	0.77	0.00	0.23						
Wheeler	0.83	0.00	0.17						
All Groups				0.43 (0.35)	11.77 (0.31)	596.13 (30.58)	345.75 (16.90)	−9.79 (0.40)	34.5 (3.05)
All sites	0.84	0.10	0.06						
Diversity statistics									
Lineage H_e	0.052	0.051	0.105						
Allelic richness	1.28	1.42	1.57						
Admixture (Q_{max})	0.494	0.878	0.891						

Note: Allelic richness (using rarefaction techniques for unequal sample sizes) and expected heterozygosity (H_e) of all individual trees assigned to the three STRUCTURAMA lineages (Lineage 1 = 517 individuals, Lineage 2 = 64 individuals, and Lineage 3 = 34 individuals) calculated in FSTAT. Admixture within lineage calculated in STRUCTURE (Pritchard et al. 2000).

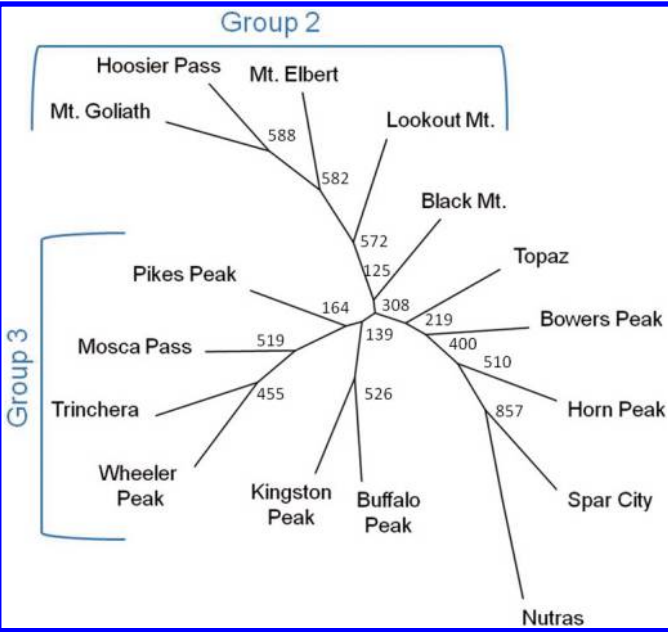
Fig. 4. Locations and genetic lineage assignments of 16 *Pinus aristata* sites sampled for genetic variation across the core range of the species in Colorado and New Mexico. Individual tree lineage assignments from STRUCTURAMA (Huelsenbeck and Andolfatto 2007) results are based on a model of $K = 3$ genetic lineages (determined as ΔK from Evanno et al. (2005) and Richards et al. (2009)).



0.075; Oline et al. 2000; *P. longaeva* $H_e = 0.327$; Hiebert and Hamrick 1983). Although the overall genetic diversity of *P. aristata* is relatively low, the populations are significantly structured. Population divergence, or isolation, is higher for *P. aristata* ($F_{ST} = 0.131$) compared with other long-lived perennials (mean

$G_{ST} = 0.076$; Hamrick and Godt 1990) or *Pinus* species in general (mean $G_{ST} = 0.065$; Hamrick et al. 1992) but comparable with other endemic or narrowly distributed long-lived woody perennials (mean $G_{ST} = 0.141$ and 0.124 , respectively; Hamrick et al. 1992). Differentiation between sites within mountain ranges indicates limited gene flow within a

Fig. 5. Phenogram of 16 populations of *Pinus aristata* (based on Nei's (1972) genetic distance using the neighbor-joining method). The numbers at each node reflect the output from the bootstrapping from which the phenogram was constructed. Groups are based on proportions of STRUCTURAMA lineage assignment proportions (see Table 4).



range and genetic drift within sites (Lewandowska-Sabat et al. 2010).

The configuration of populations on the landscape affects gene flow among populations, even for species with similar life history traits. Species such as *P. aristata* that occupy high mountaintop locations separated from one another by deep valleys can form a fragmented distribution of isolated populations. This strong mountain island effect allows populations to evolve largely independently by the action of drift and selection (Oline et al. 2000) and likely contributes to the population differentiation that we observed in *P. aristata*. In addition to geographic distance resulting in isolation, population substructuring can also be a consequence of a mating system where pollen may be widely distributed but an asynchrony of megastrobilus receptivity may prevent gene flow among nearby populations (Schuster et al. 1989). It is common to see significant variation in seed cone production among stands in *P. aristata* within a year (A.W. Schoettle, personal observation). A similar genetic pattern was observed in patchy populations of *Abies* species in southern Mexico and Guatemala (Aguirre-Planter et al. 2000). However, the degree of population differentiation in *P. aristata* may also be reduced by occasional long-distance wind dispersal of *P. aristata* seed or bird-assisted seed dispersal by Clark's Nutcracker (Torick et al. 1996).

In addition to the species' patchy alpine distribution, its extreme longevity may play a critical role in the observed degree of genetic differentiation. This role may be the product of a complex interaction between age structure, mating system, and migration. At the core of this interaction is the effective population size that determines the rate of random genetic drift. In the case of *P. aristata*, age-structured, over-

lapping generations and slow demographic turnover may reduce the overall effective population size by increasing the potential for biparental inbreeding (Engen et al. 2005). Mitigating these effects are the long-distance pollen dispersal common in wind-pollinating species and the sustained contribution of long-lived individuals to this gene flow. The calculation of the effective population size, while simple in concept, becomes quite complex in practice. Without detailed information about age-specific vital rates and their variation over time, it is difficult to attribute a definitive role of this species' unique longevity to the magnitude of differentiation. It is clear, however, that the distribution of genetic diversity and differentiation of *P. aristata* are influenced by the interplay between geography and slow demographic turnover.

Some variation in genetic diversity for *P. aristata* was associated with geographic gradients. Genetic diversity patterns were significantly correlated with longitude and elevation and for latitude in some analyses (Figs. 2 and 3). Likewise, the lineage assignments are mostly grouped together within mountain ranges and most discrepancies are within the central and northern areas of the range. A classic "isolation-by-distance" pattern emerged with some sites within the central area of the core distribution having high genetic similarity, while northern and southeastern sites were more distinct (Fig. 3). There appears to be an area of historically greater gene flow and genetic continuity encompassing the central zone of the core distribution. The high-elevation parks among the mountain peaks support more contiguous populations of *P. aristata* in this area and may permit more gene flow via stepping-stone pollen or seed dispersal among elevations across the landscape (see Schuster et al. 1989). Greater genetic diversity was apparent in the southeastern and eastern areas of the *P. aristata* core distribution (Tables 2 and 4; Fig. 2a) and these sites grouped together on the neighbor-joining tree based on Nei's (1972) genetic distance as well as in their lineage assignments (Figs. 4 and 5). The western sites (Spar City, Nutras Creek, and Bowers Peak) also grouped together and the sites with more historic gene flow, i.e., Topaz, Bowers Peak, Buffalo Peak, and Black Mountain, are in the central areas of the phenogram.

Paleoecology and genetic studies propose that other species that currently comprise the subalpine ecosystems of the Southern Rocky Mountains migrated from refugia on the eastern plains westward to the mountains after the last glacial maximum (Wells and Stewart 1987). Our data are consistent with this hypothesis for *P. aristata*. Most of the genetic diversity in *P. aristata* occurs within sites located on the eastern aspect of the eastern mountain ranges of the *P. aristata* core distribution (i.e., sites in the northern Front Range and the east side of the southern Sangre de Cristo Mountains) (Fig. 4). We propose that these areas are glacial refugia or areas that were within postglacial colonization routes for *P. aristata* as the range expanded west and northward after glacial retreat. Genetic variation is expected to be greatest in the refugial areas and decrease in the direction of migration as the time since founding decreases (Cwynar and MacDonald 1987). The leading edge expansion hypothesis of Hewitt (2000) could be entertained where several events of long-distance dispersal from the eastern front led to a loss of alleles and greater homozygosity at some of the far western sites (i.e., Spar City and Nutras) (Fig. 4), leading to the observed

longitudinal gradient in diversity (Fig. 2). This model of expansion is also associated with the production of large areas of homogeneity that persist and increase in time and space (Hewitt 2000), all in accordance with the greater diversity in the east and the genetically continuous area in the center of the *P. aristata* core range. However, a genetic diversity study alone, such as presented here, cannot test this hypothesis; complementary paleoecology studies and applications of other genetic techniques are needed to infer population histories (i.e., Betancourt et al. 1991; Mitton et al. 2000).

Natural selection may also have contributed to the development of the pattern of genetic variation observed across climatic gradients for *P. aristata*. Even for molecular markers that primarily assess neutral variation, some loss of diversity or private alleles can be associated with different habitats or strong directional selection (e.g., Kim et al. 2003). A range of habitat types occur within the core distribution of *P. aristata* (Baker 1992; Ranne et al. 1997) and we have defined some climate differences between the sites with different tree assignment proportions (Table 4). Sites towards the north-eastern and southeastern edges of the *P. aristata* core distribution have very different lineage ratios from others including trees assigned to Lineage 3 (Table 4; Fig. 4). In addition, the genetic diversity and allelic richness of individuals in Lineage 3 are higher than in Lineages 1 and 2 (Table 4). These sites are the warmest and wettest compared with the other sites (Table 4) due to the North American Monsoon and Front Range upslope rains during the growing season at more southern and eastern sites, respectively. These populations may be the most viable under a warming climate.

We explored possible explanations of how multiple distinct lineages can coexist within a single population (e.g., sites that group into Groups 2 and 3). One possibility is that the trees with lower admixture and higher allelic richness and heterozygosity (i.e., assigned to Lineages 2 and 3) may be the oldest and possibly more closely related to a glacial refugia population, while the trees in the more admixed lineage (Lineage 1) are in younger age classes. We did not find any significant patterns to support this hypothesis, as the largest, and presumably oldest, trees partitioned into the various lineages similarly as all of the trees, by site or over the all sites. This implies that either the historical signal of the founder population could not be detected or the largest trees were not the oldest. Alternatively, individuals of different lineages may have different environmental tolerances such that annual variation in environmental conditions may slightly favor establishment of different lineages in different years over the long colonization periods for this species. Jump et al. (2006) found evidence for differences in gene frequencies among *Fagus sylvatica* L. trees that varied predictably with the temperature of the year of tree establishment. Dendrochronology studies of the establishment dates of *P. aristata* trees assigned to different lineages within a site could help address this hypothesis. Likewise, fine-scale adaptation to microhabitats may also contribute to within-population diversity of lineages, although no obvious relationships with aspect or slope within a site among lineages emerged (data not shown). Alternatively, premating phenological barriers (differences in flowering synchrony) among the lineages could also contribute to maintaining their distinction within a site (Field et al.

2011). Further field observations and tests are needed to address these and other alternative hypotheses.

In summary, genetic diversity of *P. aristata* is low with significant population structure. The patterns of genetic diversity are probably due to a combination of geographic, life history, and postglacial migration processes. The species has a narrow range and its life history traits enable the species to occupy high mountaintop locations forming a fragmented distribution. This strong mountain island effect likely contributes to the population differentiation that we observed in *P. aristata*. Further population differentiation may be reduced by the sustained contribution of long-lived individuals to local and long-distance gene flow and the mixed-age stand structure resulting from the species' prolonged colonization dynamic. Genetic diversity was associated with the geographic location of the population with greater diversity in the east than in the west and at higher elevation than lower. Evidence for three lineages was detected and they were not distributed across the species' core distribution evenly. The most diverse lineages were located in the warmer and wetter southeastern and eastern sites and we hypothesize that they comprise populations that may be more closely related to glacial refugia. The distribution of genetic diversity and substructuring of *P. aristata* may be an outcome of a combination of restricted gene flow due to geographic and phenological isolation, random processes of genetic drift, life history, natural selection, and postglacial migrations. This characterization of the within- and among-population genetic diversity and structure of *P. aristata* provides a foundation for further research to define the contribution of each of these processes to the observed genetic patterns for this long-lived species.

Management implications

Genetic diversity provides the foundation for adaptation to changing environments (Aitken et al. 2008). Restricted gene flow among populations (moderate F_{ST}) may limit migration of genes or genotypes to new suitable habitats as the climate continues to change. The combination of low genetic diversity, moderate population structure, and a very protracted regeneration dynamic (Coop and Schoettle 2009) may lead to local extirpation of populations and reduced genetic diversity of the species through the loss of rare alleles as they are challenged by the direct and indirect effects of a changing climate and invasion by white pine blister rust. Furthermore, if climate suitable for *P. aristata* no longer exists on some mountains, genetic diversity of the species may be further reduced by the loss of the more genetically diverse high-elevation populations. However, the species' extreme tree longevity may enable groups of mature trees to persist, in the absence of disturbance, well after genotypes are maladapted for successful seedling establishment on the site (Hamrick 2004); this may already be the case for some stands with very little recent regeneration. Old trees have similar genetic diversity to the overall population and may serve as a reservoir of diversity from which to collect seed and pollen for the near future and may therefore warrant protection from mountain pine beetle.

Artificial regeneration with white pine blister rust resistant seedling stock may be needed to sustain populations of *P. aristata* as the white pine blister rust fungus continues to

spread (Schoettle and Snieszko 2007). These plantings also provide an opportunity for adaptive management to a changing climate. The moderate among-population variation observed in our study would suggest that seed exchange among populations of *P. aristata* by management should be conservative. However, until common garden studies quantify adaptive differentiation among sites, a mix of seed sources from both warmer and cooler sites for outplanting at cooler sites may be prudent in a warming climate. This approach would also increase the genetic diversity of the planting stock, which may also be beneficial. Physiological characterization of *P. aristata* populations will define to what extent temperature and (or) moisture conditions are selective forces for potential local adaptation. Projections of future moisture availability in mountainous areas are very uncertain and given that the distribution of *P. aristata* reflects an apparent association with the North American Monsoon, more climate research is needed before defining future suitable habitat and considering assisted migration of this species beyond its current distributional limits.

Protection of genetic diversity both within and among populations is essential for species with more disjunct populations (Falk et al. 2006). To capture the genetic diversity for *ex situ* conservation of *P. aristata*, we recommend making collections from as many populations as practical across the range, due to the moderate population structure. Areas of high genetic diversity, such as the high-elevation sites and the southeastern and eastern areas of the species' distribution that have greater heterozygosity and frequencies of rare alleles, should be prioritized for greater sampling intensity and conservation. The areas of greater genetic uniformity, in central Colorado where greater gene flow appears to have occurred historically, can be sampled with lower intensity for gene conservation.

Acknowledgements

We would like to thank Bob Westfall for assistance with the initial statistical analyses at NFGEL, Mary Frances Mahalovich and Andy Bower for advice on sampling, Sarah Schmeer for excellent field assistance, the laboratory staff at NFGEL for isozyme analyses, and the thoughtful comments of the Associate Editor and two anonymous reviewers. This research was funded, in part, by a grant from the USDA Forest Service National Fire Plan program to A.W. Schoettle.

References

- Aguirre-Planter, E., Furnier, G.R., and Eguiarte, L.E. 2000. Low levels of genetic variation within and high levels of genetic differentiation among populations of species of *Abies* from southern Mexico and Guatemala. *Am. J. Bot.* **87**(3): 362–371. doi:10.2307/2656632. PMID:10718997.
- Aitken, S.N., Yeaman, S., Holliday, J.A., Wang, T., and Curtis-McLane, S. 2008. Adaptation, migration or extirpation: climate change outcomes for tree populations. *Evol. Appl.* **1**(1): 95–111. doi:10.1111/j.1752-4571.2007.00013.x.
- Baker, W.L. 1992. Structure, disturbance, and change in the bristlecone pine forests of Colorado. *Arct. Alp. Res.* **24**(1): 17–26. doi:10.2307/1551316.
- Bentz, B.J., Régnière, J., Fettig, C.J., Hansen, E.M., Hayes, J.L., Hicke, J.A., Kelsey, R.G., Negrón, J.F., and Seybold, S.J. 2010. Climate change and bark beetles of the western United States and Canada: direct and indirect effects. *Bioscience*, **60**(8): 602–613. doi:10.1525/bio.2010.60.8.6.
- Betancourt, J.L., Schuster, W.S., Mitton, J.B., and Anderson, R.S. 1991. Fossil and genetic history of a pinyon pine (*Pinus edulis*) isolate. *Ecology*, **72**(5): 1685–1697. doi:10.2307/1940968.
- Blodgett, J.T., and Sullivan, K.F. 2004. First report of white pine blister rust on Rocky Mountain bristlecone pine. *Plant Dis.* **88**(3): 311. doi:10.1094/PDIS.2004.88.3.311A.
- Bower, A.D., and Aitken, S.N. 2007. Mating system and inbreeding depression in whitebark pine (*Pinus albicaulis* Engelm.). *Tree Genet. Genomes*, **3**(4): 379–388. doi:10.1007/s11295-007-0082-4.
- Bower, A.D., McLane, S.C., Eckert, A., Jorgensen, S., Schoettle, A.W., and Aitken, S. 2011. Conservation genetics of high elevation five-needle white pines. In *The Future of High-Elevation, Five-Needle White Pines in Western North America: Proceedings of the High Five Symposium*. Edited by R.E. Keane, D.F. Tomback, M.P. Murray, and C.M. Smith. USDA For. Serv. Proc. RMRS-P-63. pp. 98–117. Online at http://www.fs.fed.us/rm/pubs/rmrs_p063.html.
- Brown, P.M., and Schoettle, A.W. 2008. Fire and stand history in two limber pine (*Pinus flexilis*) and Rocky Mountain bristlecone pine (*Pinus aristata*) stands in Colorado. *Int. J. Wildland Fire*, **17**(3): 339–347. doi:10.1071/WF06159.
- Brunstein, F.C., and Yamaguchi, D.K. 1992. The oldest known Rocky Mountain bristlecone pines (*Pinus aristata* Engelm.). *Arct. Alp. Res.* **24**(3): 253–256. doi:10.2307/1551666.
- Coop, J.D., and Schoettle, A.W. 2009. Regeneration of Rocky Mountain bristlecone pine (*Pinus aristata*) and limber pine (*Pinus flexilis*) three decades after stand-replacing fires. *For. Ecol. Manage.* **257**(3): 893–903. doi:10.1016/j.foreco.2008.10.034.
- Cwynar, L.C., and MacDonald, G.M. 1987. Geographical variation of lodgepole pine in relation to population history. *Am. Nat.* **129**(3): 463–469. doi:10.1086/284651.
- Earl, D.A. 2009. Structure Harvester v0.3. Available from http://users.soe.ucsc.edu/~dearl/software/struct_harvest [accessed 15 November 2010].
- Engen, S., Lande, R., and Saether, B.-E. 2005. Effective size of a fluctuating age-structured population. *Genetics*, **170**(2): 941–954. doi:10.1534/genetics.104.028233. PMID:15834152.
- Evanno, G., Regnaut, S., and Goudet, J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol. Ecol.* **14**(8): 2611–2620. doi:10.1111/j.1365-294X.2005.02553.x. PMID:15969739.
- Falk, D.A., Richards, C.M., Montalvo, A.M., and Knapp, E.E. 2006. Chapter 2: Population and ecological genetics in restoration ecology. In *Foundations of restoration ecology*. Edited by D.A. Falk, M.A. Palmer, and J.B. Zedler. Island Press, Washington, D. C. pp. 14–44.
- Felsenstein, J. 1993. PHYLIP (Phylogeny Inference Package) version 3.5c. Distributed by the author. Department of Genetics, University of Washington, Seattle, Wash.
- Field, D.L., Ayre, D.J., Whelan, R.J., and Young, A.G. 2011. The importance of pre-mating barriers and the local demographic context for contemporary mating patterns in hybrid zones of *Eucalyptus aggregate* and *Eucalyptus rubida*. *Mol. Ecol.* **20**(11): 2367–2379. doi:10.1111/j.1365-294X.2011.05054.x. PMID: 21375638.
- Goudet, J. 1995. FSTAT (version 1.2): a computer program to calculate *F*-statistics. *J. Hered.* **86**(6): 485–486.
- Guerrant, E.O., Havens, K., and Maunder, M. (Editors). 2004. *Ex situ* plant conservation: supporting species survival in the wild. Island Press, Washington, D.C.
- Hamrick, J.L. 2004. Response of forest trees to global environmental

- changes. *For. Ecol. Manage.* **197**(1–3): 323–335. doi:10.1016/j.foreco.2004.05.023.
- Hamrick, J.L., and Godt, M.J.W. 1990. Allozyme diversity in plant species. *In* Plant population genetics, breeding, and genetic resources. *Edited by* A.H.D. Brown, M.T. Clegg, A.L. Kahler, and B.S. Weir. Sinauer Associates, Inc., Sunderland, Mass. pp. 43–63.
- Hamrick, J.L., and Godt, M.J.W. 1996. Effects of life history traits on genetic diversity in plant species. *Philos. Trans. R. Soc. B Biol. Sci.* **351**(1345): 1291–1298. doi:10.1098/rstb.1996.0112.
- Hamrick, J.L., Godt, M.J.W., and Sherman-Broyles, S.L. 1992. Factors influencing levels of genetic diversity in woody plant species. *New For.* **6**(1–4): 95–124. doi:10.1007/BF00120641.
- Hewitt, G.M. 2000. The genetic legacy of the quaternary ice ages. *Nature*, **405**(6789): 907–913. doi:10.1038/35016000. PMID:10879524.
- Hiebert, R.D., and Hamrick, J.L. 1983. Patterns and levels of genetic variation in Great Basin bristlecone pine, *Pinus longaeva*. *Evolution*, **37**(2): 302–310. doi:10.2307/2408339.
- Howell, B., Burns, K.S., Kearns, H.S.J., Witcosky, J.J., and Cross, F. J. 2006. Biological evaluation of a model for predicting presence of white pine blister rust in Colorado based on climatic variable and susceptible white pine species distribution. *USDA For. Serv. Biol. Eval.* R2-06-04. Online at http://www.fs.usda.gov/Internet/FSE_DOCUMENTS/fsbdev3_039457.pdf.
- Huelsenbeck, J.P., and Andolfatto, P. 2007. Inference of population structure under a Dirichlet process model. *Genetics*, **175**(4): 1787–1802. doi:10.1534/genetics.106.061317. PMID:17237522.
- Jump, A.S., Hunt, J.M., Martínez-Izquierdo, J., and Peñuelas, J. 2006. Natural selection and climate change: temperature-linked spatial and temporal trends in gene frequency in *Fagus sylvatica*. *Mol. Ecol.* **15**(11): 3469–3480. doi:10.1111/j.1365-294X.2006.03027.x. PMID:16968284.
- Kim, M.-S., Brunsfeld, S.J., McDonald, G.I., and Klopfenstein, N.B. 2003. Effect of white pine blister rust (*Cronartium ribicola*) and rust-resistance breeding on genetic variation in western white pine (*Pinus monticola*). *Theor. Appl. Genet.* **106**(6): 1004–1010. PMID:12671747.
- Lewandowska-Sabat, A.M., Fjellheim, S., and Rognli, O.A. 2010. Extremely low genetic variability and highly structured local populations of *Arabidopsis thaliana* at higher latitudes. *Mol. Ecol.* **19**(21): 4753–4764. doi:10.1111/j.1365-294X.2010.04840.x. PMID:20887360.
- Lewis, P.O., and Zaykin, D. 2001. Genetic Data Analysis: computer program for the analysis of allelic data. Version 1.0 (d16c). Free program distributed by the authors over the internet from <http://bjdnerror1.wo.com.cn:8080?HOST=lewis.eeb.uconn.edu&R=/lewishome/software.html> [accessed 20 December 2010].
- McWethy, D.B., Gray, S.T., Higuera, P.E., Littell, J.S., Pederson, G., Ray, A.J., and Whitlock, C. 2010. Climate and terrestrial ecosystem change in the U.S. Rocky Mountains and Upper Columbia Basin: historical and future perspectives for natural resource management. *Nat. Res. Rep. NPS/GRYN/NRR-2010/260*. National Park Service, Fort Collins, Co. Online at: <http://www.nature.nps.gov/publications/nrpm/nrr.cfm#2010>.
- Mitton, J.B., Kreiser, B.R., and Latta, R.G. 2000. Glacial refugia of limber pine (*Pinus flexilis* James) inferred from the population structure of mitochondrial DNA. *Mol. Ecol.* **9**(1): 91–97. doi:10.1046/j.1365-294x.2000.00840.x. PMID:10652078.
- Nei, M. 1972. Genetic distance between populations. *Am. Nat.* **106**(949): 283–292. doi:10.1086/282771.
- Oline, D.K., Mitton, J.B., and Grant, M.C. 2000. Population and subspecific genetic differentiation in the foxtail pine (*Pinus balfouriana*). *Evolution*, **54**(5): 1813–1819. PMID:11108608.
- Peakall, R., and Smouse, P.E. 2006. GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. *Mol. Ecol. Notes*, **6**(1): 288–295. doi:10.1111/j.1471-8286.2005.01155.x.
- Petit, R.J., El Mousadik, A., and Pons, O. 1998. Identifying populations for conservation on the basis of genetic markers. *Conserv. Biol.* **12**(4): 844–855. doi:10.1046/j.1523-1739.1998.96489.x.
- Pritchard, J.K., Stephens, M., and Donnelly, P. 2000. Inference of population structure using multilocus genotype data. *Genetics*, **155**(2): 945–959. PMID:10835412.
- Ranne, B.M., Baker, W.L., Andrews, T., and Ryan, M.G. 1997. Natural variability of vegetation, soils, and physiography in the bristlecone pine forests of the Rocky Mountains. *Great Basin Nat.* **57**: 21–37.
- Rehfeldt, G.E. 2006. A spline model of climate for the western United States. *USDA For. Serv. Gen. Tech. Rep. RMRS-GTR-165*.
- Richards, C.M., Antolin, M.F., Reilley, A., Poole, J., and Walters, C. 2007. Capturing genetic diversity of wild populations for *ex situ* conservation: Texas wild rice (*Zizania texana*) as a model. *Genet. Resour. Crop Evol.* **54**(4): 837–848. doi:10.1007/s10722-006-9167-4.
- Richards, C.M., Volk, G.M., Reilley, A.A., Henk, A.D., Lockwood, D.R., Reeves, P.A., and Forsline, P.L. 2009. Genetic diversity and population structure in *Malus sieversii*, a wild progenitor species of domesticated apple. *Tree Genet. Genomes*, **5**(2): 339–347. doi:10.1007/s11295-008-0190-9.
- Rosenberg, N.A., Pritchard, J.K., Weber, J.L., Cann, H.M., Kidd, K., Zhivotovsky, L.A., and Feldman, M.W. 2002. Genetic structure of human populations. *Science*, **298**(5602): 2381–2385. doi:10.1126/science.1078311. PMID:12493913.
- Salzer, M.W., Hughes, M.K., Bunn, A.G., and Kipfmüller, K.F. 2009. Recent unprecedented tree-ring growth in bristlecone pine at the highest elevations and possible causes. *Proc. Natl. Acad. Sci. U.S.A.* **106**(48): 20348–20353. doi:10.1073/pnas.0903029106. PMID:19918054.
- Schoettle, A.W. 2004. Ecological roles of five-needle pine in Colorado: potential consequences of their loss. *In* Breeding and genetic resources of five-needle pines: growth, adaptability, and pest resistance. *Edited by* R.A. Snieszko, S. Samman, S.E. Schlarbaum, and H.B. Kriebel. *USDA For. Serv. Proc. RMRS-P-32*. pp. 124–135. Online at http://www.fs.fed.us/rm/pubs/rmrs_p032/rmrs_p032_124_135.pdf.
- Schoettle, A.W., and Snieszko, R.A. 2007. Proactive intervention to sustain high-elevation pine ecosystems threatened by white pine blister rust. *J. For. Res.* **12**(5): 327–336. doi:10.1007/s10310-007-0024-x.
- Schuster, W.S.F., Alles, D.L., and Mitton, J.B. 1989. Gene flow in limber pine: evidence from pollination phenology and genetic differentiation along an elevational transect. *Am. J. Bot.* **76**(9): 1395–1403. doi:10.2307/2444563.
- Torick, L.L., Tomaback, D.F., and Espinoza, R. 1996. Occurrence of multi-genet tree clusters in “wind-dispersed” pines. *Am. Midl. Nat.* **136**(2): 262–266. doi:10.2307/2426730.
- USDA Forest Service. 2003. National Forest Genetic Electrophoresis Laboratory Standard Operating Procedures. NFGEL, U.S. Department of Agriculture, Forest Service, Placerville, Calif. Online at <http://www.fs.fed.us/forestmanagement/silviculture/nfgel/documents/protocols/SOP2003.pdf>.
- Walters, C., Volk, G.M., and Richards, C.M. 2008. Genebanks in the post-genomic age: emerging roles and anticipated uses. *Biodiversity*, **9**: 68–71.

- Wells, P.V., and Stewart, J.D. 1987. Cordilleran-boreal taiga and fauna on the central Great Plains of North America, 14,000–18,000 years ago. *Am. Midl. Nat.* **118**(1): 94–106. doi:10.2307/2425632.
- Westfall, R.D., and Conkle, M.T. 1992. Allozyme markers in breeding zone designation. *New For.* **6**(1–4): 279–309. doi:10.1007/BF00120649.