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Mitochondrial DNA haplotype distribution patterns in *Pinus ponderosa* (Pinaceae): Range-wide evolutionary history and implications for conservation[1]

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- *Premise of the study:* Ponderosa pine (*Pinus ponderosa* Douglas ex P. Lawson & C. Lawson) exhibits complicated patterns of morphological and genetic variation across its range in western North America. This study aims to clarify *P. ponderosa* evolutionary history and phylogeography using a highly polymorphic mitochondrial DNA marker, with results offering insights into how geographical and climatological processes drove the modern evolutionary structure of tree species in the region.
- *Methods:* We amplified the mtDNA *nad1* second intron minisatellite region for 3,100 trees representing 104 populations, and sequenced all length variants. We estimated population-level haplotypic diversity and determined diversity partitioning among varieties, races and populations. After aligning sequences of minisatellite repeat motifs, we evaluated evolutionary relationships among haplotypes.
- *Key results:* The geographical structuring of the 10 haplotypes corresponded with division between Pacific and Rocky Mountain varieties. Pacific haplotypes clustered with high bootstrap support, and appear to have descended from Rocky Mountain haplotypes. A greater proportion of diversity was partitioned between Rocky Mountain races than between Pacific races. Areas of highest haplotypic diversity were the southern Sierra Nevada mountain range in California, northwestern California, and southern Nevada.
- *Conclusions: Pinus ponderosa* haplotype distribution patterns suggest a complex phylogeographic history not revealed by other genetic and morphological data, or by the sparse paleoecological record. The results appear consistent with long-term divergence between the Pacific and Rocky Mountain varieties, along with more recent divergences not well-associated with race. Pleistocene refugia may have existed in areas of high haplotypic diversity, as well as the Great Basin, Southwestern United States/northern Mexico, and the High Plains.

Key words: Migration; minisatellite; mitochondrial DNA; phylogeography; *Pinus ponderosa*; *Pinus washoensis*; Pleistocene; polymorphism

Ponderosa pine (*Pinus ponderosa* Douglas ex Lawson) is the most broadly distributed pine species of the Western Hemisphere (Critchfield and Little, 1966), where it has considerable ecological and economic importance (Oliver and Ryker, 1990). Its distribution encompasses montane environments from British Columbia to southern California and from northern Montana to Arizona and New Mexico, and extends east into the plains of Nebraska, Oklahoma, and Texas (Little, 1971; Farjon, 1984). The species exhibits extensive morphological variation across its range (Weidman, 1939; Squillace and Silen, 1962; Wells, 1964; Read, 1980), suggesting that it may be in the early stages of differentiation into multiple species (Wang, 1977; Jaramillo-Correa et al., 2009), or at least into multiple distinct regional lineages. Geographic patterns of needle and cone morphology, growth traits, monoterpene content, and isozyme content (reviewed in Conkle and Critchfield, 1988), as well as DNA sequence variation (Gernandt et al., 2009), have created confusion about evolutionary relationships within the *P. ponderosa* complex. Meanwhile, the near absence of a paleoecological record for the species during the Pleistocene (Van Devender et al., 1987; Anderson, 1989; MacDonald et al., 1998), obscures important phylogeographic processes that have influenced the evolutionary history of the ponderosa pine complex.

We used information from a highly polymorphic mitochondrial DNA minisatellite region to assess the processes governing the geographic distributions of genealogical lineages within ponderosa pine. Such organelle noncoding DNA regions are particularly useful for studies of plant intraspecific phylogeography (Pleines et al., 2009), which can help clarify the location of origin, vicariance factors, and migrational processes that have produced patterns of variation across the range of tree species (Jaramillo-Correa et al., 2009). Given the broad environmental and geographic distribution of *P. ponderosa* across

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western North America, a better understanding of its evolutionary history and phylogeography should also offer insights into how biogeographical and climatological processes drove the modern evolutionary structure of other species in the region (e.g., see Spellman and Klicka, 2006).

Ponderosa pine is generally considered to encompass at least two varieties. The western variety, *P. ponderosa* var. *ponderosa* Laws. (Pacific ponderosa pine), exists from the mountains of southern California to British Columbia and inland to Idaho and Montana; this variety has open, plume-like foliage, a low proportion of two-needle fascicles, larger cones and seeds than the eastern variety, and more flexible needles. The eastern variety, *P. ponderosa* var. *scopulorum* Engelm. (Rocky Mountain ponderosa pine), exists in the interior Rocky Mountains from Montana to Arizona and New Mexico; this variety has compact and brush-like foliage, moderate to high proportions of two-needle fascicles, with needles that are relatively stiff (Conkle and Critchfield, 1988). The two make contact in a transition zone near the Continental Divide in west-central Montana (Critchfield, 1984; Latta and Mitton, 1999; Johansen and Latta, 2003). Morphological variation, meanwhile, has led some taxonomists to further subdivide the Pacific and Rocky Mountain varieties (Smith, 1977; Conkle and Critchfield, 1988). As many as five different divisions have been described within the species complex, three within the Pacific variety (Pacific Coast, North Plateau, and Washoe), and two within the Rocky Mountain variety (northern Rocky Mountains and Southwestern) (Fig. 1).

For pines and other conifers, the distributions of mitochondrial DNA (mtDNA) haplotypes are informative for detecting ancient events of differentiation and dispersal long after gene flow has erased the signature of different refugial origins within nuclear and chloroplast genes (Sinclair et al., 1999; Mitton et al., 2000b). Unlike paternally inherited chloroplast DNA (cpDNA), mtDNA is maternally inherited in most pine species (Wagner et al., 1987; Neale and Sederoff, 1989; Dong and Wagner, 1993, 1994) and is therefore dispersed only by seed movement, while cpDNA is dispersed by wind-borne pollen movement, generally across larger distances (Neale et al., 1986; Strauss et al., 1989; Dong and Wagner, 1994). As a result of reduced gene flow, earlier geographic structure is probably retained longer for seed-borne mtDNA markers than for cpDNA and nuclear markers that are dispersed among populations by both pollen and seed flow (Petit et al., 1993; Ennos, 1994). Mitochondrial DNA was more spatially structured than cpDNA in a single population of ponderosa pine, for example, supporting the hypothesis that seed movement is restricted while cpDNA is homogenized by pollen dispersal (Latta et al., 1998). Additionally, the mtDNA genome effectively behaves as a single haploid gene (Birky et al., 1989), which means that initial genetic differentiation resulting from drift among refugial populations is probably greater than for diploid nuclear markers. Different refugial origins among populations are therefore more likely to be detectable with mtDNA than with nuclear markers (Sinclair et al., 1999).

We quantified mitochondrial DNA haplotype variation across the range of ponderosa pine to clarify the evolutionary history and phylogeography of the *P. ponderosa* complex, and to assess whether patterns of differentiation within the species correspond with subspecific taxa. Specifically, we conducted a range-wide assessment of ponderosa pine phylogeography using haplotypes from the second intron of the mitochondrial genome *nad1* gene (Mitton et al., 2000a) in 3,100 trees representing 104 populations. Our specific objectives were to: (1) sequence the nucleotides within size variant haplotypes of the *nad1* intron to understand the repeat structure of this minisatellite region, and to align the haplotypes' minisatellite tandem repeat motif sequences to estimate evolutionary relationships among them; (2) assess whether haplotype diversity within populations is associated with latitude, longitude, and elevation, which could indicate patterns of post-Pleistocene movement; (3) infer phylogeographic processes within the ponderosa pine complex based on patterns of haplotype distribution across the range of the species and on the evolutionary relationships among the haplotypes; and (4) test whether mtDNA haplotype distributions and the partitioning of diversity among varieties and races correspond with proposed divisions of *P. ponderosa* into subordinate taxa. The results should be useful for the management and conservation of this widespread and important species in the face of climate change and other threats.

MATERIALS AND METHODS

***Sample collection and DNA extraction*—**This analysis encompassed foliage samples collected between 2001 and 2012 from a total of 3,113 trees representing 104 populations across the range of ponderosa pine within the United States (Table 1, Fig. 2). With only two exceptions, at least 20 trees were sampled in each population; most populations encompassed at least 30 sampled trees. One of these exceptions, the Grass Creek population (#75) in Wyoming, is a disjunct population containing only 10 mature trees and every tree was included in the study. Legacy trees and populations (those established before 1900 with natural regeneration and no evidence of reforestation planting activities) were the focus of collection. The sampled population in the Wah Wah Mountains in western Utah (#52) includes the oldest known living ponderosa pine, estimated to be approximately 930 yr old (Kitchen, 2010). Populations were not included in the study if they had undergone reforestation activities. If possible, samples were limited to mature trees, and, where possible, sampled trees were at least 100 meters apart to increase the probability of sampling the entire range of a population's genetic composition. Two populations were included that have been identified by Critchfield and Little (1966) as Washoe pine (*Pinus washoensis* H.L. Mason & Stockw.) yet appear to include trees with *P. ponderosa*-like morphology: Babbitt Peak (#17) in California and Mount Rose (#18) in Nevada, the location of the type specimen for Washoe pine. Two other populations, Saguaro National Park (#66) and Whitetail Trail (#71) both in Arizona, are *P. ponderosa* populations existing within the range of *P. arizonica* Engelm., but not co-occurring with it. Ten samples of Coulter pine (*P. coulteri* D. Don), collected from a provenance trial at the Institute of Forest Genetics in Placerville, California, were included to serve as an outgroup. Coulter pine appears to fall into a clade closely related to ponderosa pine and its nearest relatives (Willyard et al., 2009). One branch tip 3-5 inches in length was collected per tree, each containing a dormant terminal bud and healthy needles attached to the stem. Samples were kept cool, but not frozen, until they were shipped to the National Forest Genetics Laboratory (NFGEL) in Placerville, California, for DNA extraction and sequencing. Genomic DNA for all samples was extracted from the needle samples using the DNEasy 96 Plant Kit (Qiagen, Chatsworth, California, USA). DNA concentrations were determined using a Gemini XPS Microplate Spectrofluorometer (Molecular Devices, Sunnyvale, California, USA) with PicoGreen dsReagent (Invitrogen, Carlsbad, California, USA).

***Mitochondrial minisatellite generation*—**The minisatellite region in the second intron in the *nad1* mitochondrial genome (Demesure et al., 1995) was used as a DNA marker. Such minisatellites are intermingled arrays of variant repeats, ranging from 10 bp to more than 100 bp, which can be highly polymorphic, possess a relatively low mutation rate, and contain relatively simple intra-allelic turnover mechanisms (Bonhomme et al., 2007). The *nad1* intron region has served as a useful molecular marker for population genetic surveys because it contains size variants that reveal population structure across the range of *Pinus ponderosa* and *P. flexilis* E. James (Latta et al., 1998; Latta and Mitton, 1999; Mitton et al., 2000b; Mitton et al., 2000a; Johansen and Latta, 2003). Pine sequences available for the *nad1* region in Genbank (*Pinus cembra* L. AF160261, *P. densata* Mast. AF440388, *P. pinaster* Aiton AJ509804-AJ509806, *P. ponderosa* AF231325, *P. pumila* (Pallas) Regel AF227463, *P. sibirica* Du Tour AF160260,

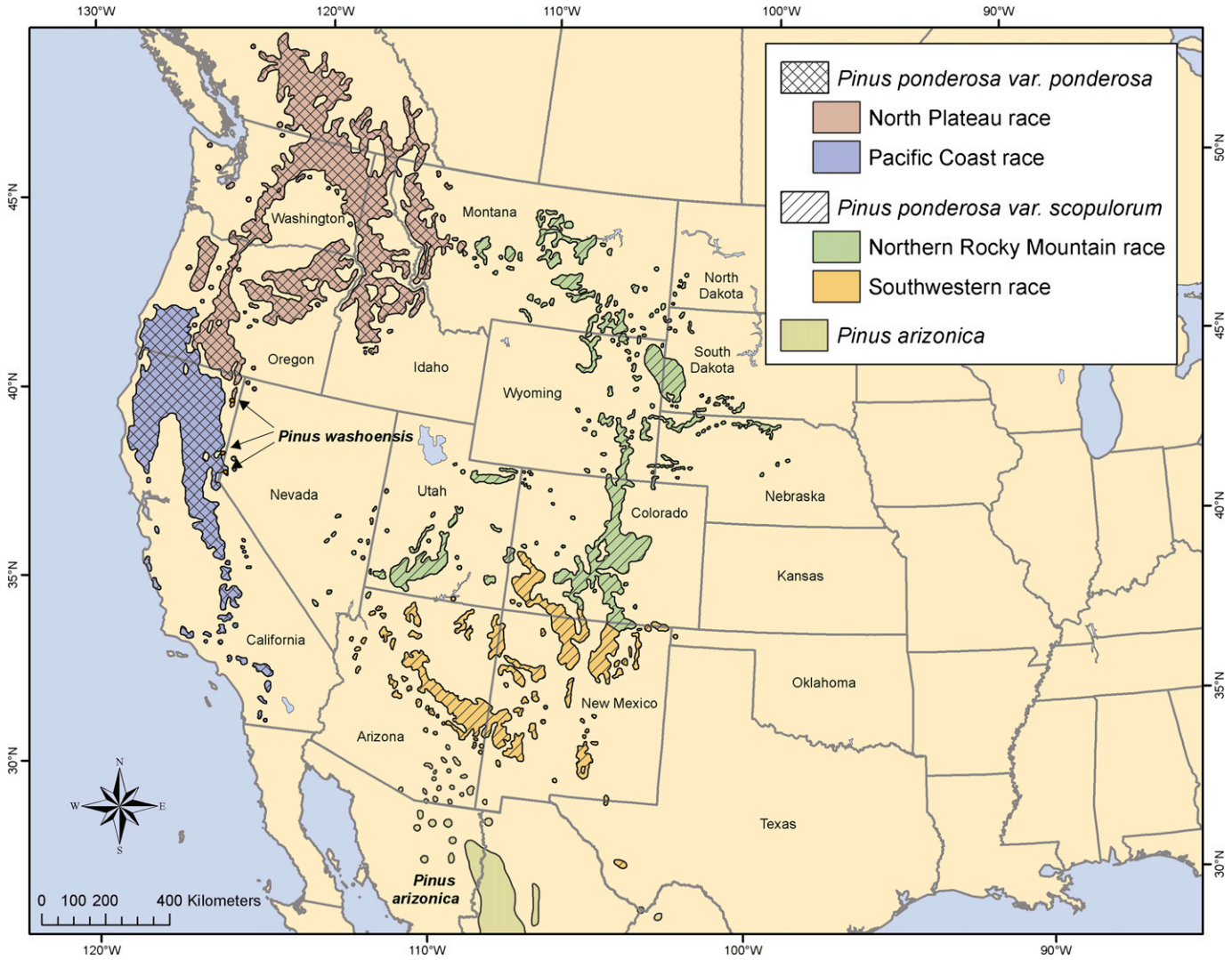

Fig. 1. Geographical distributions of *Pinus ponderosa* var. *ponderosa* and *P. ponderosa* var. *scopulorum* and of the two races described within each variety, in addition to the locations of *P. washoensis* and *P. arizonica*. Boundaries between races are approximate.

P. sylvestris L. AJ223312, *P. tabuliformis* Carrière AF440384, and *P. yunnanensis* Franch. AF440385-AF440387) were downloaded and aligned using the GeneDoc software (Nicholas et al., 1997). These alignments were used to design forward and reverse PCR primers GGGGCTTATGGGTGAGCAAT (nad1-in2_F2) and CTCTGAATTGACGAATGCCG (nad1-in2_R2), respectively, using the computer program GeneRunner v3.04 (Hastings Software, Hudson, New York, USA).

PCR amplification was achieved for 3,100 of the 3,113 ponderosa pine samples, and all 10 of the Coulter pine samples, in 25 µl reaction volumes that included 10 mm TRIS HCl pH 8.3, 50 mm KCl, 1.5 mm $MgCl_2$, 200 µm of each dNTP, 1 µm of each primer, 12 ng of DNA template, and 0.5 units of HotStar *Taq* DNA Polymerase from Qiagen (Valencia, California, USA). Following HotStart *Taq* activation (94°C for 15 min), PCR amplification involved denaturation at 94°C for 20 s, annealing for 30 s, and extension for 2 min. The annealing temperature during the initial 10 cycles was lowered from 65° to 60° by 0.5° every second cycle. An additional 30 cycles of amplification were performed upon reaching the final annealing temperature (60°C) followed by a final extension at 72° for 10 min. The forward primer was labeled with a fluorescent tag for visualization on an ABI Prism 3130xl capillary electrophoresis system (Applied Biosystems, Foster City, California, USA) following a 1:50 dilution of amplification product. Visual checks were performed on all electrophoresis products.

Length variants were directly sequenced in both the forward and reverse directions for 228 samples (7.4% of the total), including at least one representative of each length variant from each population and at least 5.6% of the total of each variant. PCR products were purified using the QIAquick PCR Purification kit (Qiagen) following the manufacturer's instructions. Sequence analysis was performed with an ABI-3730 Genetic Analyzer using ABI BigDye Terminator v3.1 Cycle Sequencing (Applied Biosystems, Foster City, California, USA) and the PCR amplification primers. ABI chromatograms were aligned and edited using the Sequencher v.4.5 software (Gene Codes Corporation, Ann Arbor, Michigan, USA).

DNA sequencing revealed the presence of a pair of variants differing by a single base pair in length (442 bp and 443 bp), which were indistinguishable by prior fragment analysis on the ABI Prism 3130xl, and the presence of a second pair of variants differing by three base pairs (408 bp and 411 bp), which were difficult to distinguish. DNA sequences were used to identify a restriction enzyme digest pattern that could be used to screen samples from both pairs of variants. Restriction digests were performed on all samples that were determined to contain a 442 bp or 443 bp size variant via ABI visualization, and on all samples from central and southern Nevada and California containing a 408 bp or 411 bp size variant. Amplified products were cleaned using the QIAquick PCR Purification Kit (Qiagen), and digested for two hours at 60°C with BstNI (New England BioLabs, Ipswich, Massachusetts, USA). Digested products were separated on 1.4% agarose gels stained with GelRed (Biotium, Hayward, California, USA) and visualized under UV light. Discrete bands of 260 bp / 95 bp and 240 bp / 95 bp were observed in the 442 bp and 443 bp

TABLE 1. Location, sample size, coordinates, elevation, source, number of haplotypes (*nh*), haplotype diversity (*H*), and haplotype composition (Haps) for the 104 *Pinus ponderosa* populations included in the mtDNA analysis.

Pop.	Location, state	n	Latitude (°N)	Longitude (°W)	Elevation (m)	Source	*nh*	*H*	Haps
1	Larabee Valley, CA	30	40.43	−123.68	738	a	2	0.46	5,9
2	Applegate Valley West, OR	33	42.29	−123.28	533	a	2	0.11	5,8
3	Eugene, OR	26	43.92	−123.13	313	a,b	2	0.07	5,8
4	Hoadley Peaks North, CA	30	40.69	−122.75	1,413	a	1	0.00	5
5	Monarch Mountain, CA	31	40.59	−122.57	747	c	2	0.17	1,5
6	Fort Lewis, WA	30	47.11	−122.50	91	d	1	0.00	5
7	Klamath River Canyon, OR	30	42.06	−122.08	1,017	a	3	0.56	1,5,9
8	Pothole, OR	30	42.95	−121.88	1,413	e	3	0.24	1,5,8
9	Lava Beds National Monument, CA	30	41.70	−121.57	1,551	c	1	0.00	1
10	Henry W. Coe State Park, CA	32	37.19	−121.55	908	f	1	0.00	9
11	Lassen Volcanic National Park, CA	34	40.53	−121.54	1,679	c	2	0.11	1,5
12	Modoc, OR	30	42.97	−121.39	1,836	e	1	0.00	8
13	Adobe West, OR	30	42.00	−121.00	1,481	a	1	0.00	1
14	Wenatchee, WA	30	47.60	−120.43	1,036	e	1	0.00	8
15	Lost Forest, OR	30	43.38	−120.32	1,370	a	2	0.06	1,8
16	Madeline Plains, CA	30	40.86	−120.11	1,722	a	1	0.00	1
17	Babbitt Peak, CA	30	39.61	−120.11	2,624	e	1	0.00	1
18	Mount Rose, NV	30	39.34	−119.88	2,317	e	1	0.00	1
19	Yosemite National Park, CA	30	37.54	−119.65	1,260	c	2	0.36	1,5
20	Sheldon National Wildlife Refuge, NV	30	41.83	−119.64	2,002	g	1	0.00	1
21	Potato Flat, OR	30	43.77	−119.45	1,615	e	1	0.00	1
22	Sequoia National Monument, CA	30	36.77	−119.09	1,376	e	3	0.41	1,5,10
23	Red Wash Canyon, NV	33	38.40	−119.07	2,028	e	1	0.00	1
24	Alamo Mountain, CA	31	34.67	−118.96	2,243	e	1	0.00	2
25	Umatilla, OR	30	44.97	−118.87	1,158	e	1	0.00	1
26	Wassuk Range, NV	30	38.41	−118.68	1,988	e	1	0.00	1
27	Bishop Creek, CA	30	37.30	−118.53	1,972	e	1	0.00	10
28	Bobsled, OR	30	45.69	−118.26	1,401	e	1	0.00	1
29	Charlton Flats, CA	30	34.30	−118.01	1,634	e	1	0.00	2
30	Wallowa-Whitman, OR	29	45.13	−117.68	1,341	e	1	0.00	1
31	Strawberry Peak, CA	30	34.23	−117.21	1,815	e	1	0.00	2
32	Mineral Ridge, ID	30	47.62	−116.68	733	a	1	0.00	8
33	Nez Perce, ID	30	45.45	−115.92	1,268	e	1	0.00	1
34	Lee Canyon, NV	30	36.33	−115.66	2,420	h	3	0.29	2,3,4
35	Kyle Canyon, NV	30	36.26	−115.61	2,137	e	1	0.00	4
36	Red Rock Canyon, NV	30	36.12	−115.48	1,189	a	2	0.32	2,4
37	Grant Range, NV	29	38.44	−115.44	2,174	a	1	0.00	7
38	Sheep Range High, NV	30	36.59	−115.22	2,314	g	2	0.32	3,4
39	Sheep Range Low, NV	30	36.63	−115.11	1,981	g	2	0.50	2,4
40	Seaman Range, NV	27	38.07	−115.10	2,271	a	2	0.42	3,7
41	North Pahroc Range, NV	29	37.68	−114.95	2,118	a	1	0.00	3
42	Glacier National Park, MT	30	48.83	−114.32	1,108	c	1	0.00	8
43	Snake Range, NV	30	39.01	−114.25	2,313	c	1	0.00	7
44	Bitterroot, MT	30	45.83	−114.14	1,676	e	1	0.00	1
45	Beaver Dam State Park, NV	30	37.51	−114.09	1,608	i	1	0.00	7
46	Mormon Gulch, UT	32	37.97	−114.03	2,225	j	1	0.00	7
47	Toms Creek, UT	29	39.87	−113.88	2,438	a	1	0.00	7
48	Hualapai Mountains, AZ	30	35.06	−113.87	1,868	a	1	0.00	3
49	Black Rock Mountains, AZ	31	36.80	−113.75	2,103	a	2	0.44	3,7
50	Steamboat Mountain, UT	32	38.09	−113.69	1,951	a	1	0.00	7
51	Big Hole National Battlefield, MT	31	45.65	−113.66	1,952	c	1	0.00	1
52	Wah Wah Mountains, UT	24	38.60	−113.55	2,467	a	1	0.00	7
53	Dunningan Gulch, MT	26	46.96	−113.53	1,118	a	1	0.00	8
54	Blackfoot River-Dupont, MT	25	47.00	−113.32	1,155	a	1	0.00	8
55	Sinbad Spring, UT	30	39.38	−113.32	2,387	a	1	0.00	7
56	Brown's Gulch, MT	32	46.35	−113.27	1,804	a	1	0.00	8
57	Big Hole River/Valley, MT	36	45.64	−112.96	1,612	a	1	0.00	1
58	Marcum Mountains, MT	30	46.94	−112.89	1,301	a	1	0.00	8
59	Ranch Canyon, UT	31	38.40	−112.81	2,164	a	1	0.00	7
60	Kaibab, AZ	27	35.83	−112.08	1,981	e	1	0.00	3
61	Boulder Mountains, MT	30	46.51	−112.03	1,701	a	1	0.00	8
62	Coconino, AZ	29	35.00	−111.67	2,134	e	1	0.00	3
63	Big Belt Mountains, MT	30	46.33	−111.27	1,473	a	1	0.00	6
64	Little Belt Mountains, MT	30	46.89	−111.20	1,676	a	1	0.00	6
65	Henry Mountains, UT	30	38.07	−110.84	2,417	a,j	2	0.42	3,7
66	Saguaro National Park, AZ	12	32.15	−110.52	1,859	c	1	0.00	3
67	White Rocks Canyon, UT	30	40.62	−109.94	2,274	e	1	0.00	3
68	Bridger Creek, MT	30	45.65	−109.77	1,349	a	1	0.00	6

TABLE 1. Continued.

Pop.	Location, state	n	Latitude (°N)	Longitude (°W)	Elevation (m)	Source	*nh*	*H*	Haps
69	Book Cliffs, UT	30	39.56	−109.41	2,109	a	1	0.00	3
70	Judith Mountains, MT	30	47.13	−109.36	1,463	a	1	0.00	6
71	Whitetail Trail, AZ	20	32.03	−109.32	1,920	c	1	0.00	3
72	Apache-Sitgreaves, AZ	70	34.05	−109.31	2,444	e	1	0.00	3
73	Douglas Mountain, CO	30	40.58	−108.68	2,195	a	2	0.23	3,7
74	Little Rocky Mountains, MT	30	47.90	−108.63	1,224	a	1	0.00	6
75	Grass Creek, WY	10	43.90	−108.58	1,750	a	1	0.00	3
76	Boggy Draw, CO	31	37.51	−108.48	2,310	e	1	0.00	3
77	Sawmill Mesa, CO	30	38.49	−108.40	2,560	e	2	0.14	3,6
78	Square S Gulch, CO	31	39.97	−108.29	1,980	a	1	0.00	3
79	Shepard Recreation Site, MT	30	46.06	−108.29	1,031	a	1	0.00	6
80	Big Horn Mountain, WY	32	44.13	−107.38	1,866	a	2	0.06	3,6
81	San Juan-Pagosa, CO	29	37.30	−107.10	2,408	e	1	0.00	3
82	Eight-Mile, CO	30	37.17	−106.98	2,438	e	2	0.06	3,6
83	Seminoe Mountains, WY	30	42.17	−106.94	2,186	a	1	0.00	6
84	Sparks, CO	30	37.27	−106.90	2,377	e	1	0.00	3
85	Billy Creek, WY	29	44.10	−106.85	2,327	a	1	0.00	3
86	Lower Willow Creek, CO	33	37.66	−106.60	2,675	e	2	0.42	3,6
87	Rawlins, WY	30	41.02	−106.48	2,469	e	2	0.39	3,6
88	Stove Gulch, WY	30	43.25	−106.38	1,687	h,a	2	0.46	3,6
89	Four Elk, CO	30	38.90	−106.21	2,659	a	1	0.00	6
90	North Inlet, CO	30	40.26	−105.80	2,675	c	1	0.00	6
91	Ojito, NM	30	36.14	−105.72	2,466	e	1	0.00	3
92	Red Feather Lakes, CO	30	40.79	−105.58	2,552	e	2	0.06	3,6
93	Walker Canyon Bottom, NM	30	32.97	−105.54	2,257	e	2	0.39	2,3
94	Wild Basin, CO	30	40.22	−105.54	2,505	c	2	0.29	3,6
95	Mescalero Apache Reservation, NM	30	33.07	−105.38	1,942	k	2	0.12	2,3
96	Deer Haven, CO	30	38.61	−105.38	2,458	a	2	0.06	3,6
97	Casper, WY	32	42.37	−105.35	1,951	e	1	0.00	6
98	Vedauwoo, WY	30	41.19	−105.29	2,353	e	1	0.00	3
99	Stonewall, CO	30	37.14	−105.05	2,486	h	1	0.00	3
100	Rampart Range, CO	20	38.95	−104.94	2,848	e	2	0.42	3,6
101	Eddy, NM	30	32.15	−104.76	1,813	e	1	0.00	2
102	Black Hills, SD	35	44.20	−103.82	1,945	e	1	0.00	6
103	Black Mesa West, OK	30	36.83	−102.96	1,393	l	1	0.00	3
104	Niobrara Valley Preserve, NE	30	42.78	−100.08	755	m	1	0.00	6

Note: Sources: a, Bureau of Land Management; b, City of Eugene, Oregon; c, National Park Service; d, Department of Defense; e, USDA Forest Service; f, California State Parks; g, Fish and Wildlife Service; h, private land; i, Nevada Division of State Parks; j, Utah School Institutional Trust Land Administration; k, Bureau of Indian Affairs; l, Oklahoma School Land Trust; m, The Nature Conservancy.

variants, respectively. Discrete bands of 260 bp / 95 bp and 240 bp / 95 bp were observed in the 408 bp and 411 bp variants, respectively.

***Statistical analyses*—**We used GenAlEx 6.41 (Peakall and Smouse, 2006) to calculate two population-level estimates of haplotypic diversity, number of haplotypes (*nh*) and haplotype diversity (*H*), which is equivalent to expected heterozygosity in diploid data (Nei, 1987). We also determined the mean population value of these statistics in a nested fashion: (1) across the species; (2) separately within *Pinus ponderosa* var. *ponderosa* and *P. ponderosa* var. *scopulorum*; and (3) separately within two subgroups within *P. ponderosa* var. *ponderosa* (North Plateau and Pacific Coast) and two within *P. ponderosa* var. *scopulorum* (Northern Rocky Mountain and Southwestern) (Fig. 1). While these subgroups have been given varietal or even species rank by some authors, we refer to them as races for simplicity. Because only two populations of Washoe pine were sampled, they were included with the North Plateau race, to which Washoe pine is closely related (Critchfield, 1984; Rehfeldt, 1999a). We used SAS 9.2 (SAS Institute, 2008) to test for statistically significant correlations among haplotype diversity statistics and population latitude, longitude, and elevation, across the species, and within varieties and races.

We also used GenAlEx 6.41 (Peakall and Smouse, 2006) to conduct a three-tiered hierarchical analysis of molecular variance (AMOVA) (Excoffier et al., 1992; Huff et al., 1993) to determine the partitioning of diversity among populations, regions, and populations within regions. This analysis was repeated three times, once across the entire range with the Pacific and Rocky Mountain varieties treated as regions, and once each for the two varieties, with the races within the varieties treated as regions. The significance of the variance components was determined with 999 permutations. This AMOVA method generates Φ_{PT}, which estimates the proportion of the total variance that is partitioned among populations (Excoffier et al., 1992; Huff, 1997) and is analogous to F_{ST} (Peakall and Smouse, 2006).

Minisatellite DNA regions possess the unique properties of rapid simple mutation and complex internal structure that, when correctly aligned, make them highly informative for the quantification of molecular divergence between variants and for phylogenetic analysis (Bonhomme et al., 2007). DNA sequence alignment methods cannot be used for sequences of minisatellites consisting of imperfect tandem repeats with variation in the sequence of the repeat motifs because such methods count only point mutations and disregard the main source of sequence divergence within minisatellites, tandem duplication, and contraction events, which are much more frequent than point mutations (Bonhomme et al., 2007). The alignment of motif sequences within minisatellite regions should account for tandem duplication and tandem contraction of a motif in addition to insertion, deletion and substitution of motifs (Berard and Rivals, 2003). Because the *nad1* minisatellite region in ponderosa pine contains several multiple-motif duplications (e.g., "AB"), we aligned the sequences of motifs manually.

We constructed two sets of matrices to evaluate the evolutionary relationships among the haplotypes based on differences between pairs of aligned motif sequences. These matrices could then be used as input for standard phylogenetic reconstruction (Berard et al., 2006; Bonhomme et al., 2007). For one pairwise matrix, each difference was given a weight of 1, which has the effect of treating all mutations as equally likely. For our second pairwise difference matrix, we assigned specific penalty values to different types of mutation events based on their likelihood relative to a simple contraction or duplication of a motif in a series of tandem motifs. We gave these simple events a value of 1 whether the contraction or duplication was of a single motif or of a block of motifs in a series of those blocks (specifically, "BA"). We assigned the value of

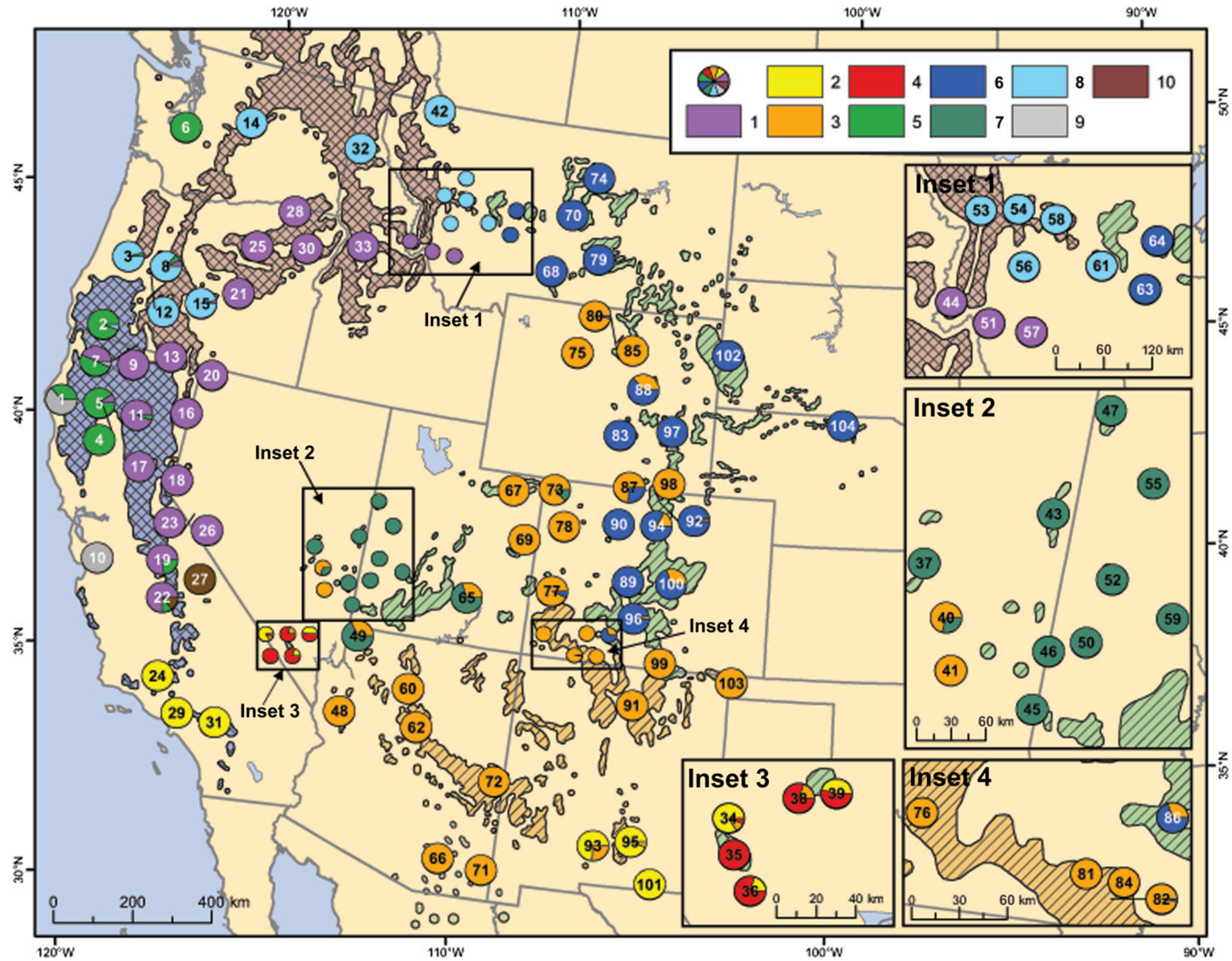

Fig. 2. Mitochondrial DNA haplotype distributions in *Pinus ponderosa*. See Table 1 for population information and Fig. 1 for identification of varieties and races. Note that *P. ponderosa* populations 66 and 71 occur within the range of *P. arizonica*, but do not co-occur with that species. Lines from some pie charts point to the actual locations of those populations.

a substitution of one motif for another based on the number of differences in the DNA sequences between the two motifs (Table 2). The substitution of B with H was given a value of 6 because the two motifs are separated by five point mutations and a single 20 bp insertion in H that we treated as a single difference. (The multiple substitutions of H for B in Haplotypes 9 and 10 seem likely to have occurred as a single event, rather than multiple times, and were therefore counted only once.) The deletion of the D motif, which occurred in three haplotypes, was given a value of 10 to reflect the hypothesis that this motif was lost only once and not as a result of multiple events in different haplotypes; this value was determined based on the number of mutations required for the preceding C motif to become a D motif (a single repeat of the C motif followed by nine point mutations). Finally, the deletion of a string of multiple motifs at the beginning of Haplotype 10 was given a value of 1 because this probably occurred as a single event in a single haplotype.

Table 2. Nucleotide sequences of the eight repeat motifs that together constitute each of the *Pinus ponderosa* mtDNA haplotypes; nucleotide positions that differ from the sequence of the A motif are highlighted.

ID	Nucleotide Sequence
A	c c c t c a c c - a - t a t g a a t a g t - g a g t g t g c t t a c g c a
B	c c c t c **t** c c - **-** - t **-** **g** g a **c** **-** a g t **c** g a g t g t g c t **a** a c g c a
C	c c c t c a c c - a - t a t g a a t a g t - g a g t g **-** **-** **-** **-** **-** a c **-** **-** **-**
D	c c c t c a c c - a - t **c** **g** g a a t a g t - g a g t g **-** g c t **c** **-** c g c **-**
E	c c c t c **t** c c - **t** - t **-** **g** g a **c** **-** a g t **c** g a g t g t g c t t a c g c a
F	c c c t c **-** **-** **-** - **-**
G	c c c t **a** a **a** **t** - a **a** t a t **a** **g** **g** **g** **-**
H	c c c t c **t** c c **c** **c** **t** **c** **c** **t** **c** **t** **c** **a** **a** **a** **t** **a** **a** **g** **t** **g** **t** **a** **a** **c** **c** **a** **a** a **g** g **-** **c** **-** a g t **c** g a g t g t g c t **a** a c g c a

The neighbor-joining (NJ) algorithm (Saitou and Nei, 1987) is a robust method for constructing trees from evolutionary distances (Mihaescu et al., 2009) that performs a heuristic search of tree space where each step is guided by a criterion of minimizing tree branch length, but, like some other phylogenetic methods, does not explore all possible topologies and has no guarantee of finding the "best" tree (Gascuel and Steel, 2006). Therefore, for both our genetic difference matrices, we used the R 2.14.1 program Analyses of Phylogenetics and Evolution (APE) (Paradis et al., 2004) to build a FastME (Desper and Gascuel, 2002) tree. This employed a "greedy minimum evolution" (GME) algorithm, which is related to NJ, and used the balanced nearest neighbor interchange tree-swapping algorithm to search for the best topology. This approach has been found to yield a significant improvement over NJ and other distance-based algorithms both in terms of computational performance and topological accuracy (Desper and Gascuel, 2002). We also used APE to establish confidence estimates for each of the nodes in our two GME phylograms, based on 1,000 bootstrap replicates.

In phylogeographic studies, the correct estimation of allele or haplotype relationships is particularly important, and network approaches have been thought superior to tree-building methods for inferring gene or locus relationships (Pleines et al., 2009). Traditional phylogenetic analyses, however, have been found to perform as well or better than such network methods in the accurate reconstruction of known evolutionary relationships (Cassens et al., 2005; Woolley et al., 2008; Salzburger et al., 2011). While network methods have the ability to display potential ambiguity of their inferences in a single graphical representation by allowing reticulations, or multiple connections between haplotypes (Woolley et al., 2008), they do not allow for the weighting of specific motif differences that was required by the ponderosa pine motif sequence data. A more appropriate approach was to combine the motif sequence data with the GME tree data to generate haplotype genealogies using the program Haploviewer (Salzburger et al., 2011). Haplotype genealogies are unrooted trees, displayed as acyclic graphs, with labeled leaves that refer directly to haplotypes that have been sampled from the population, and with edges that are integers greater than zero that represent discrete mutational steps between haplotypes (Salzburger et al., 2011). Haploviewer uses the alignment (of the motif sequences within the minisatellite regions, in this case) and the topology information from the GME phylograms to determine the haplotype genealogy and the number of mutational steps between haplotypes.

RESULTS

***Minisatellite structure*—**We detected 10 size variants of the second intron in the *nad1*mitochondrial region from the sampled ponderosa pine trees, ranging in length from 342 bp to 584 bp (Table 3). Three additional size variants were present in Coulter pine, which were 576 bp, 678 bp and 744 bp in length. Nucleotide sequencing of multiple individuals from each length variant confirmed that each is a different haplotype, and that the *nad1* second intron is a minisatellite region containing differing arrangements of eight related sequence motifs. These motifs range in length from 5 to 54 base pairs, with most approximately 30 base pairs in length (Table 2). Motif H contains an insertion of 20 nucleotides not contained in any of the other motifs, while motifs F and G encompass substantial deletions relative to the other motifs. Separate from these multiple-sequence insertions and deletions, 23 aligned nucleotide locations show single-base substitutions, deletions, or additions relative to repeat motif A.

When aligned, most of the haplotypes began with a B motif, followed by one to eight A motifs, followed by three to five repetitions of the "BA" combinations; within this region, two ponderosa pine haplotypes (Haplotypes 4 and 7) and all three Coulter pine haplotypes contained a repeat of a single B or A motif (Table 3). These are likely the result of a tandem duplication of the single motif, or a duplication of the "BA" combination followed by a later deletion of either the A or B motif. The overall motif sequence ended with B, C, D, G, E, and F. Within these closing motifs, seven of the ponderosa pine haplotypes and all of the Coulter pine haplotypes included the D motif, while three (Haplotypes 2, 4, and 7) did not. In two other haplotypes (Haplotypes 9 and 10), all or most of the B motifs were substituted with H motifs. Most of the first half of the sequence was missing in Haplotype 10 relative to the other motif sequences, most likely the result of one or more deletion events.

***Haplotype geographic distributions*—**Geographical structuring is apparent in the distribution of the 10 ponderosa pine haplotypes (Fig. 2). The four most widely distributed were Haplotype 1, which extended around the western and northern edges of the Great Basin; Haplotype 3, which extended throughout much of the interior of the range from southern Arizona to northern Wyoming; Haplotype 5, which extended from central California to Washington; and Haplotype 6, which also occurred in the interior West, from southern Colorado to northern Montana.

Haplotypes 1, 6, and 3 appear to correspond, in size and location, with Haplotypes A, C, and D, respectively, as described in Latta and Mitton (1999) and Johansen and Latta (2003). Haplotypes 3 and 6 co-occurred in nine populations in the central Rocky Mountains. Haplotype 3 also co-occurred, in other populations, with Haplotypes 2, 4, and 7. Haplotype 2 was located in three relatively small but widely dispersed locations, in southern

TABLE 3. Fragment sizes, minisatellite sizes, GenBank accession numbers, and frequency of occurrence for the *Pinus ponderosa* and *P. coulteri* (outgroup) haplotypes, and alignment of motifs in each.

Haplotype	Fragment size (bp)	Minisatellite size (bp)	GenBank accession	Freq.	Alignment of minisatellite repeat motifs
1	569	408	JX826417	583	B A . . . . . B A B A B A . . . . B C D G E F
2	572	411	JX826418	216	B A A . . . . . B A B A B A . . . . B C . G E F
3	603	442	JX826419	779	B A A . . . . . B A B A B A . . . . B C D G E F
4	604	443	JX826420	96	B A A . . . . . B . B A B A B A . . B C . G E F
5	635	474	JX826421	160	B A . . . . . B A B A B A B A . . B C D G E F
6	637	476	JX826422	515	B A A A . . . . . B A B A B A . . . . B C D G E F
7	674	513	JX826423	319	B A A A A . . . . B A B A B A . A . . B C . G E F
8	701	540	JX826424	344	B A . . . . . B A B A B A B A B A B C D G E F
9	745	584	JX826425	53	B A . . . . . H A H A H A H A . . H C D G E F
10	503	342	JX826426	34	. . . . . H A H A . . H C D G E F
Coulter 1	737	576	JX826427	6	B A A A A A . . . B A B A B . B A . . B C D G E F
Coulter 2	839	678	JX826428	3	B A A A A A A A A B A B A B . B A . . B C D G E F
Coulter 3	905	744	JX826429	1	B A A A A A A A A B A B A B A B . B A B C D G E F

New Mexico in the east and in southern Nevada and California 900 km to the west. Haplotype 4 occurred only in five southern Nevada populations, mostly existing with Haplotype 2 or 3. It was fixed only in the Kyle Canyon population (#35). Haplotype 7 existed mostly in eastern Nevada, southern Utah and northwestern Arizona, but the Douglas Mountain population (#73) in northwestern Colorado also contained a small frequency of it. Haplotype 8 was limited to an arc from southern Oregon northeast into central Washington and northern Idaho and east into Montana; it appears to correspond with Haplotype B described in Latta and Mitton (1999) and Johansen and Latta (2003). We detected no intermixing of Haplotypes 1, 6, and 8 in the area of west-central Montana where they are located in close proximity. Haplotype 9 occurred in three widely dispersed populations in northern California and southern Oregon, but was fixed only in the Henry W. Coe State Park population (#10) in California. Haplotype 10, meanwhile, existed in only two populations near the southern end of the Sierra Nevada in California, and was fixed in the Bishop Creek population (#27). Six haplotypes were present in *P ponderosa* var. *ponderosa* populations, all but one present in California; five haplotypes were detected in *P. ponderosa* var. *scopulorum* populations, all but one present in Nevada. Thirty populations were polymorphic (Table 1, Fig. 2), ten *P. ponderosa* var. *ponderosa* populations (24% of the total) and 20 *P. ponderosa* var. *scopulorum* populations (32%). Four populations contained three distinct haplotypes: (a) Klamath River Canyon, Oregon (#7); (b) Pothole, Oregon (#8); (c) Sequoia, California (#22); and (d) Lee Canyon, Nevada (#34). Klamath River Canyon had the highest haplotype diversity, followed by Sheep Range Low, Nevada (#39), Larabee Valley, California (#1), Stove Gulch, Wyoming (#88), and Black Rock Mountains, Arizona (#49) (Table 1).

Populations across the range of ponderosa pine, and within *P ponderosa* var. *ponderosa* and *P. ponderosa* var. *scopulorum*, had a mean of approximately 1.33 haplotypes per population (*nh*) (Table 4). Races within *P. ponderosa* var. *scopulorum* had similar *nh* values, while the Pacific Coast race exhibited significantly higher *nh* than the North Plateau race within *P. ponderosa* var. *ponderosa*. Mean haplotype diversity (*H*), meanwhile, was higher in *P. ponderosa* var. *scopulorum* than in *P. ponderosa* var. *ponderosa*; the Northern Rocky Mountains race had significantly higher *H* than the Southwest race, while the Pacific Coast race had higher *H* than the North Plateau race. The percent of populations with multiple haplotypes followed a similar pattern. Across the species, population *H* was weakly but significantly negatively correlated with latitude (Table 4). A stronger significant correlation existed between both *H* and *nh* and longitude in *P. ponderosa* var. *ponderosa* and in the North Plateau race (i.e., higher diversity in populations farther west). A negative correlation existed between *H* and elevation in *P. ponderosa* var. *ponderosa,* and between *H* and *nh* and elevation in the Pacific Coast race. In *P. ponderosa* var. *scopulorum*, meanwhile, *nh* was moderately correlated with elevation, while *nh* and *H* were moderately negatively correlated with latitude in the Northern Rocky Mountain race.

The AMOVA results for the species (Φ_{PT} = 0.917) and for the two varieties (Φ_{PT} = 0.915 for *P. ponderosa* var. *ponderosa* and Φ_{PT} = 0.891 for *P. ponderosa* var. *scopulorum*) demonstrated that approximately 90% of the variance was partitioned among populations rather than within them (Table 5). At the species level, 64% of the genetic variance was found among populations within varieties, compared to 28% between varieties. The percent of genetic variance occurring among races was higher in *P. ponderosa* var. *scopulorum* (27%) than in *P. ponderosa* var. *ponderosa* (16%).

Relationships among haplotypes—The GME phylogram trees with and without weighting of motif sequence differences were highly consistent in their topologies (Fig. 3). Both trees clustered Haplotypes 9 and 10 with high bootstrap support; this group then clustered with Haplotype 5. This clade was subsequently grouped with Haplotype 8, with high bootstrap support; that group clustered with Haplotype 1, again with high support. Haplotype 3 was sister to this group, with high support in the tree with weighting of motif sequence differences, but without high support without the weighting. The trees also placed Haplotype 7 as a sister to all (Fig. 3A) or most (Fig. 3B) other haplotypes, followed by Haplotype 6. The two trees, however, differed in where they placed Haplotypes 2 and 4. When the loss of motif D was weighted equal to all other sequence differences, these haplotypes were placed in a sister clade to Haplotypes 3, 1, 8, 5, 9, and 10 (Fig. 3A). When the loss of motif D was given greater weight, Haplotypes 2 and 4 were grouped with Haplotype 7 (Fig. 3B), since all three haplotypes lack motif D.

The haplotype genealogies generated from these GME phylograms were also similar, with the exception of where they placed Haplotypes 2 and 4 (Fig. 4). When sequence differences are given equal weight, the GME genealogy infers that Haplotype 3 is the ancestor of Haplotype 2, which is in turn ancestor of Haplotype 4 (Fig. 4A). When the loss of motif D is given greater weight, Haplotype 7 is inferred as the ancestor of both Haplotype 2 and 4 (Fig. 4B). Haplotype 4 differs from Haplotype 2 by an extra B in the middle section of the motif sequence (likely the result of an A deletion or a B duplication) and an additional "BA" repeat (Table 2). Haplotype 7, meanwhile, has two more A repeats than Haplotypes 2 and 4 in the beginning of

TABLE 4. Population characteristics across *Pinus ponderosa*, within the Pacific (var. *ponderosa*) and Rocky Mountain (var. *scopulorum*) varieties, and within the two major races within the varieties; correlations significant at $P < 0.05$ are in bold italics.

			Population mean (SE)		Correlation with *nh*			Correlation with *H*		
Taxon	N_p	PM_p	*nh*	*H*	Latitude	Longitude	Elevation	Latitude	Longitude	Elevation
Pinus ponderosa	104	28.9	1.33 (0.05)	0.08 (0.02)	–0.173	–0.100	0.056	***-0.214***	–0.036	–0.007
Pinus ponderosa* var. *ponderosa	41	24.4	1.32 (0.10)	0.06 (0.02)	–0.182	***-0.445***	***-0.324***	–0.230	***-0.383***	–0.296
North Plateau	24	12.0	1.16 (0.10)	0.02 (0.10)	–0.226	***-0.427***	–0.178	–0.209	–0.377	–0.115
Pacific Coast	17	43.8	1.56 (0.18)	0.14 (0.05)	0.345	–0.376	***-0.560***	0.285	–0.364	***-0.544***
Pinus ponderosa* var. *scopulorum	63	31.8	1.33 (0.06)	0.09 (0.02)	–0.179	–0.015	***0.274***	–0.173	–0.088	0.209
Northern Rocky Mountains	47	34.0	1.36 (0.08)	0.11 (0.03)	***-0.369***	–0.053	0.279	***-0.383***	–0.102	0.227
Southwest	16	25.0	1.25 (0.11)	0.05 (0.03)	0.064	0.299	0.302	–0.159	0.290	0.204

Note: N_p, number of populations; PM_p, percent of populations with multiple haplotypes; *nh*, number of haplotypes; *H*, haplotype diversity.

TABLE 5. Analyses of molecular variance (AMOVAs) for (1) *Pinus ponderosa* and the Pacific and Rocky Mountain varieties, (2) *P. ponderosa* var. *ponderosa* and the races contained within, and (3) *P. ponderosa* var. *scopulorum* and the races contained within; all variance components are significant at $P = 0.001$.

Source	df	SS	MS	Variance	%	Φ	Value
Pinus ponderosa							
Among varieties	1	216.14	216.14	0.139	27.8%	Φ_{RT}	0.280
Among populations within varieties	102	964.95	9.46	0.316	63.9%	Φ_{PR}	0.885
Within populations	2995	123.58	0.04	0.041	8.3%		
						Φ_{PT}	0.917
Total	3098	1304.66		0.496	100.0%		
Pinus ponderosa var. *ponderosa*							
Among races	2	56.62	28.31	0.059	15.6%	Φ_{RT}	0.156
Among populations within races	38	329.66	8.68	0.287	75.9%	Φ_{PR}	0.899
Within populations	1194	38.38	0.03	0.032	8.5%		
						Φ_{PT}	0.915
Total	1234	424.65		0.378	100.0%		
Pinus ponderosa var. *scopulorum*							
Among races	1	92.298	92.298	0.116	26.9%	Φ_{RT}	0.269
Among populations within races	61	486.379	7.973	0.269	62.1%	Φ_{PR}	0.850
Within populations	1801	85.199	0.047	0.047	10.9%		
						Φ_{PT}	0.891
Total	1863	663.876		0.432	100.0%		

the motif sequence, and has an extra A in middle section of the sequence, likely the result of an A duplication or the loss of a B motif. Haplotype 3, on the other hand, differs from Haplotype 2 only by the deletion of the D motif.

Both genealogies infer that Haplotypes 6 and 7 share a common ancestor that is near the outgroup. Haplotype 6 is separated from Haplotype 7 by three or four steps. Haplotype 1 differs from Haplotype 6 by the apparent deletion of two A motifs in the beginning of the motif, and is related to Haplotype 3 via the deletion of a single A repeat motif. Haplotype 5 is related to Haplotype 1 via the addition of a single "BA" repeat. Haplotype 8 is related to Haplotype 5 via the addition of a second "BA" repeat, and, separately, Haplotypes 9 and 10 are related to Haplotype 5 via the switch of the B motifs to H motifs in both haplotypes and the loss of the first portion of the sequence in Haplotype 10. These results and the geographical arrangement of the haplotypes suggest that Haplotype 7 (possibly with Haplotypes 2 and 4) may belong to a lineage that is sister to the rest of *P. ponderosa*. This clade may have diverged in the interior of the current species range, with the separation of the ancestors of Haplotype 7 and Haplotype 6. The

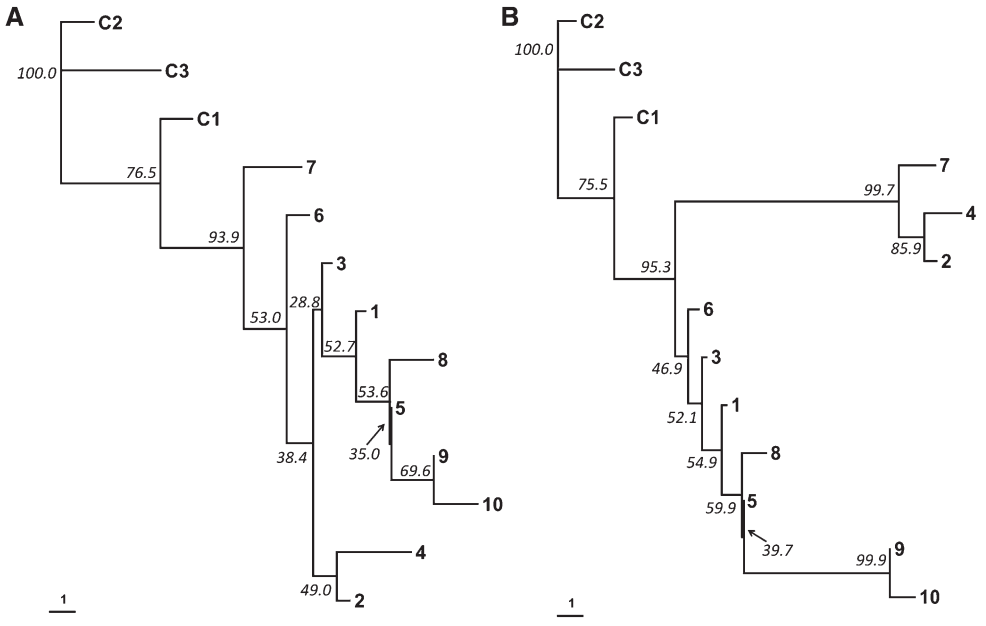

Fig. 3. Greedy minimum evolution (GME) phylograms of genetic distance between *Pinus ponderosa* haplotypes in which differences in motif sequences were given (A) equal weight and (B) varying weights. C1, C2, and C3 are the *P. coulteri* outgroup haplotypes. The values represent the percent bootstrap support for the nodes over 1,000 replicates.

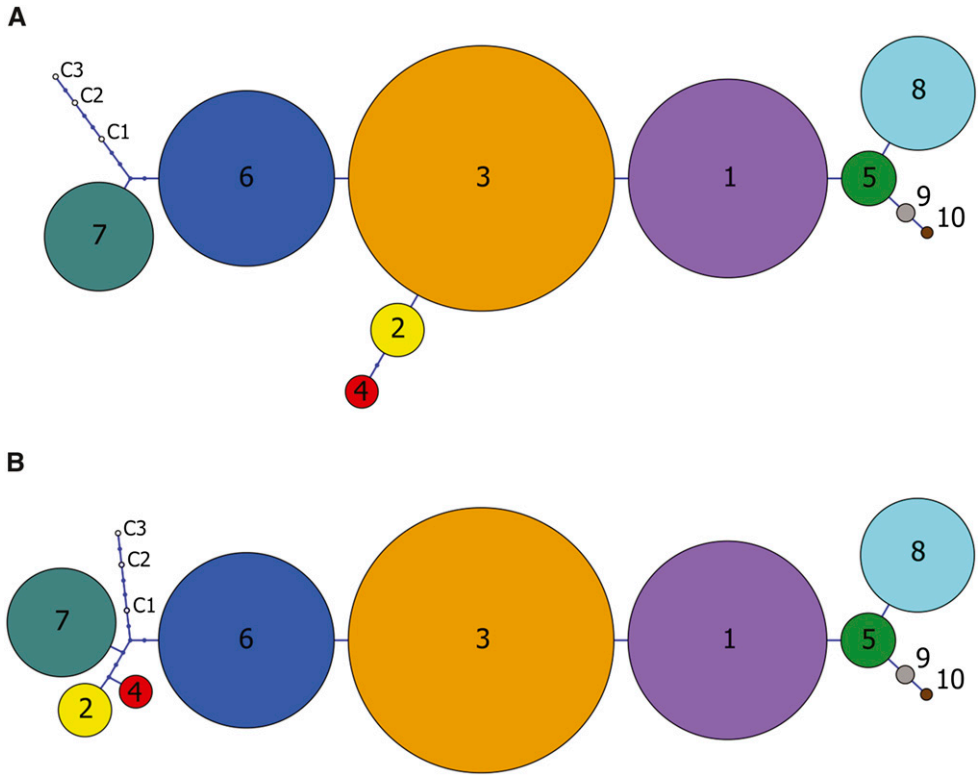

Fig. 4. Pinus ponderosa haplotype genealogies based on minisatellite motif sequence alignments and on the greedy minimum evolution (GME) phylograms in which differences in haplotype motif sequences were (A) given equal weight and (B) given varying weights. The size of each haplotype is proportional to its relative frequency in the data. (For haplotype frequencies, see Table 3.) Internodes are one mutational step. C1, C2, and C3 are the *P. coulteri* outgroup haplotypes.

ancestors of Haplotypes 6 and 3, and then those of Haplotypes 3 and 1, may have diverged later, with the remaining exterior haplotypes radiating more recently, particularly along the western edge of the *P. ponderosa* range.

DISCUSSION

The patterns of mtDNA haplotype distribution across the range of ponderosa pine and the evolutionary relationships among the haplotypes together suggest that the species has a complex phylogeographic history. Such molecular data combined with complementary paleobotanical or palynological information can provide a reasonable picture of the major geological, climatological, or ecological processes driving the modern population structure of a species (Jaramillo-Correa et al., 2009; Gugger et al., 2010). While ponderosa pine is the most widely distributed pine in western North America, the paleoecological record for the species is nearly entirely lacking from the Pleistocene (Van Devender et al., 1987; Anderson, 1989; MacDonald et al., 1998), making it a challenge to determine recent ponderosa pine phylogeographic processes with certainty. Pleistocene climatic conditions apparently restricted ponderosa pine to a geographic distribution that was much narrower than expected given its existing range (Spaulding, 1984). Inferring ponderosa pine evolutionary history must therefore rely heavily on other sources of data, such as the geographical patterns of ponderosa pine haplotype distribution and the evolutionary relationships among the haplotypes, while considering the sparse paleoecological data when possible. We can additionally compare this information to morphological and allozyme marker variation across the range of the species to assess how well the haplotype distributions correspond to proposed taxonomic divisions within the species, and to guide management of the species' genetic resources.

In the current study, the patterns of haplotype occurrence across the range of ponderosa pine, the inferred evolutionary relationships among the haplotypes, and the partition of variance between varieties are all consistent with a long-existing separation between the Pacific variety (*P. ponderosa* var. *ponderosa)* and the interior Rocky Mountain variety (*P. ponderosa* var. *scopulorum)*. A distinct division exists between the group of haplotypes detected across most of the Pacific variety range (Haplotypes 1, 5, 8, 9, and 10) and those detected within the bounds of the Rocky Mountain variety (2, 3, 4, 6, and 7) (Fig. 2). The Pacific variety haplotypes also consistently cluster together in the phylograms of genetic distance among haplotypes, forming a distinct clade (Fig. 3). Additionally, approximately 28% of the haplotypic variance in the species was detected between the two varieties (Table 5), indicating important differentiation. Such varietal differentiation was also identified in patterns of allozyme allele frequencies (Niebling and Conkle, 1990) and leaf oil terpene composition (Rudloff and Lapp, 1992), but not in patterns of xylem resin monoterpene characteristics (Smith, 1977). The amount of haplotypic variance between previously proposed varieties, however, may not be adequate to justify giving *P. ponderosa* var. *scopulorum* species rank, as suggested by the results of a chloroplast sequence analysis (Gernandt et al., 2009).

These mtDNA minisatellite results are consistent with coalescent simulations suggesting the ponderosa pine varieties have been separated for more than 10 000 generations, or more than 250 000 yr (Lascoux et al., 2004), and support the hypothesis that the main division within ponderosa pine occurred well before the last glacial maximum (18 000 yr BP). This evolutionary break between interior and coastal regions is also present in other western North American tree species, including *Pinus contorta* Douglas (Godbout et al., 2008), *Pseudotsuga menziesii* Carriere (Li and Adams, 1989; Gugger et al., 2010), *Pinus albicaulis* Engelm. (Richardson et al., 2002), *Thuja plicata* Donn ex D.Don (O'Connell et al., 2008), and *Salix melanopsis* Nutt. (Carstens et al., 2005). Within *Pseudotsuga menziesii*, Gugger et al. (2010) estimated that the coastal and Rocky Mountain varieties diverged between 4.37 million and 755 000 yr BP, a period spanning the early Pliocene to the middle Pleistocene. Given that ponderosa pine-like fossils predate the Pliocene (which began approximately 5.33 million years BP) both in California (Minnich, 2007) and in the Great Basin and Rocky Mountain regions (Axelrod, 1986), the distribution of ponderosa pine probably was such that it may have undergone intraspecies division at approximately the same time as *Pseudotsuga menziesii*, perhaps as a result of the same geological and climatological factors. The two *P. ponderosa* varieties do, in fact, have seasonally different precipitation limitations, with var. *ponderosa* requiring significant winter moisture and var. *scopulorum* needing significant summer precipitation, so adaptation to different local Quaternary or pre-Quaternary precipitation seasonality may have played a role in their differentiation (Norris et al., 2006). The division within *Pseudotsuga menziesii* may be associated with the creation of the Cascade and Sierra Nevada ranges and the subsequent xerification of the Columbia Plateau and Great Basin (Gugger et al., 2010). There is uncertainty, however, about when the Sierra Nevada uplift occurred and whether the subsidence of a Nevadaplano plateau that existed in the current Basin and Range region was a more recent event (Henry, 2009). At least two competing ideas exist about the topographic evolution of the Sierra Nevada (McPhillips and Brandon, 2012). First, geomorphic evidence such as stream incision suggests that that the Sierra Nevada uplift occurred within the last 5 million to 10 million years (Unruh, 1991; Wakabayashi and Sawyer, 2001). Second, other lines of evidence suggest that the northern Sierra Nevada acted as a steep western flank of the gradually sloping high-elevation Nevadaplano in the Oligocene (34-23 million years BP), with elevations in the Basin and Range region decreasing in the Miocene to Holocene (Cassel et al., 2012b; Cassel et al., 2012a; Chamberlain et al., 2012). Isotopic evidence suggests that the Sierra Nevada has been at approximately the same elevation since at least 16 million years BP (Chamberlain and Poage, 2000). A subsidence of the Nevadaplano, meanwhile, may have happened between about 17 million and 10 million years BP (Colgan and Henry, 2009). Some evidence suggests that both hypotheses about the Sierra Nevada uplift may be correct (McPhillips and Brandon, 2012). Regardless of the timing, it appears that important environmental changes occurred in the Sierra Nevada region in the last 2-5 million years that have driven ongoing genetic diversification (Calsbeek et al., 2003). The distribution of ponderosa pine mtDNA haplotypes and the inferred relationships among these haplotypes suggest that an ancestor of the closely related Haplotypes 1 and 3 may have existed in the current Great Basin region, and that the descendants of this haplotype may have been divided when the Sierra Nevada uplift and/or the Nevadaplano subsidence occurred.

The current spatial distribution of mtDNA haplotypes, along with limited paleoecological information, may clarify more recent evolutionary processes, including those associated with multiple glaciated and short, Holocene-like interglacial periods within the Pleistocene (Porter, 1989). The evolutionary relationships among the haplotypes within the range of *P. ponderosa* var. *ponderosa* indicate that Haplotype 1 split from Haplotype 5 by way of a single mutation, with Haplotypes 8 and 9 splitting from Haplotype 5 with separate single mutations. Haplotype 10 was then separated from Haplotype 9 by a single mutational event. A much higher level of haplotypic diversity exists in the southern, Pacific Coast race of *P. ponderosa* var. *ponderosa*, suggesting that considerable diversification occurred in this geographically and geologically complex region. The pattern of evolutionary differentiation described here could explain dramatic differences in resin monoterpene characteristics between the previously recognized North Plateau and Pacific Coast races (Smith, 1977; Sturgeon, 1979), although Haplotype 1 and, to a lesser degree, Haplotype 5 straddle the approximate boundary between the races. The distribution of ponderosa pine haplotypes in this region fits a common population distributional pattern for the Californian Floristic Province that has been detected in several species, i.e., a northern lineage that may extend into the Pacific Northwest, a restricted eastern Sierran lineage, and a southern lineage that may extend south into northwestern Mexico (Calsbeek et al., 2003; Jaramillo-Correa et al., 2009). Another example is *Pinus jeffreyi* Balfour, a close relative of *P. ponderosa* in which a strong break exists between interior Sierra and Southern California populations and coastal northwestern California populations (Furnier and Adams, 1986). The differentiation of these lineages usually predates the Quaternary, while more recent migration, hybridization, and introgression are thought to have resulted from Pleistocene glacial cycles (Jaramillo-Correa et al., 2009). This may generally be the case in *P. ponderosa* var. *ponderosa*, although Haplotypes 9 and 10 are rare with limited distributions, suggesting they diverged following more recent mutation events (Godbout et al., 2005).

Because mtDNA is maternally inherited in pines, haplotype locations may reveal post-Pleistocene routes of migration from glacial refugia via seed dispersal (Johansen and Latta, 2003). For example, Haplotypes 1, 5, and 9/10 may have found refuge in the southern Sierra Nevada, where remains of ponderosa pine date as far back as 45 000 yr BP (Cole, 1983; Anderson, 1990). Ponderosa pine at the time existed at considerably lower elevations than it currently inhabits, with the gentle western slopes of the Sierra Nevada range apparently allowing for simple downslope migration in response to climatic cooling during the Wisconsin and upslope migration during the Holocene (Anderson, 1989); such movements probably occurred multiple times in response to climatic variability, including multiple warm interstadials during that period. In the context of this climate variability, Haplotype 1 may have moved north once or more than once along the Sierra Nevada range, then skirted the western and northern edges of the Great Basin. Haplotype 5 may have moved north, once or more, along the western edge of the Sierra Nevada into northwestern California and then into Oregon and Washington. Gerson et al. (2009) propose that ponderosa pines in the Fort Lewis area of Washington (population #6) are the youngest populations in the region, given its close proximity to the Cordilleran ice shield.

Northwestern California and southwestern Oregon, the location of Pleistocene refugia for multiple species (Shafer et al., 2010), may represent another ponderosa pine refugial area. Haplotypes 5, 8, and 9 may have existed here during a recent glacial period, with Haplotype 8 possibly diverging from Haplotype 5 at that time and later dispersing north and east. In eastern Washington, ponderosa pine and lodgepole pine (*Pinus contorta* Douglas ex Loudon) had become established by the early Holocene and remained on mesic sites (Baker, 1983). A sharp rise in the percentages of diploxylon pine pollen about 8,500 yr BP records the establishment of ponderosa pine forest in the southwestern Columbia Basin of south-central Washington; with the exception of western Washington, basins in Washington and Oregon apparently were too dry and cold to support widespread forest during the most recent glacial maximum, and were instead covered with communities resembling periglacial steppe (Barnosky et al., 1987). It is possible that additional refugial areas, as yet undetected, existed for important tree species in the northwestern United States (Barnosky et al., 1987). More suitable conditions, for example, may have existed along the western edge of Washington and Oregon as the lower sea level created a coastal plain extending some 50 km farther west than that of today, and which may have had considerable precipitation (Baker, 1983).

Our understanding of the phylogeographic history of the Rocky Mountain variety of ponderosa pine, *P. ponderosa* var. *scopulorum*, is particularly constrained by the lack of paleoecological data. During the late Pleistocene, the absence of such information indicates that ponderosa pine forest was nearly or entirely absent from the northern and central Rockies, the Great Basin, the Mojave Desert, the Colorado Plateau, and the Chihuahuan Desert (Wells, 1983a; Van Devender et al., 1987; Thompson, 1990; Anderson et al., 2000). This absence is not easy to explain, given ponderosa pine's tolerance of a broad suite of climates across a latitudinal range of about 23 degrees, and given that other conifers such as *Pseudotsuga menziesii* thrived in northern parts of the Southwest during the full glacial (Betancourt et al., 1990). Because of the influence of the Laurentide Ice Sheet on atmospheric air-flow patterns, conditions in the Southwest were likely marked by lower summer temperatures and higher precipitation than present, but with less precipitation falling during the growing season (Spaulding et al., 1983; Van Devender et al., 1987; Thompson et al., 1993). In particular, cooler late summers suppressing tree growth and recruitment may help to explain the glacial-age absence of ponderosa pine in the Colorado Plateau, in addition to conditions conducive to less-frequent fires through a reduction of thunderstorm activity and lightning strikes (Betancourt, 1990; Anderson, 1993).

Ponderosa pine may have been an important component in montane forest communities south of 35° N at the height of the glaciation, and may have been most common in the Sierra Madre Occidental of northern Mexico (Betancourt et al., 1990). Patterns of genetic and monoterpene variation among ponderosa pine races and its cross compatibility with Mexican pines appear to confirm this hypothesis (Smith, 1977; Conkle and Critchfield, 1988). A handful of locations from this region have yielded fossil evidence of ponderosa pine from the late Pleistocene. These include sites in southern Arizona and southern New Mexico where *scopulorum*-type needles date from 13 500 to 14 900 yr BP, the latter being the only record of ponderosa pine east of the Continental Divide from the Wisconsin period (Anderson, 1989; Van Devender, 1990). Otherwise, packrat midden studies generally detect ponderosa pine in Arizona and New Mexico only as recently as approximately 10 000 yr BP (Cole, 1981; Smith and Betancourt, 1998; Anderson et al., 2000). Ponderosa or lodgepole pine pollen identified from the Mogollon Rim of east-central Arizona from ca. 29 000 yr BP (Anderson et al., 2000), suggests the possibility of more widespread occurrence at lower elevations (Anderson, 1989), but that pollen may have been transported from extensive distances to the south (Betancourt, 1990). The current wide longitudinal extent of Haplotypes 2 and 3, and the considerable environmental variation of the sites occupied by these haplotypes, also seem to point to extensive glacial-age distributions.

The rapid expansion of ponderosa pine into its extensive modern range represents one of the most remarkable Holocene dispersal events of a conifer in western North America (Van Devender et al., 1984). It was widespread across the Southwest by 10 000 to 9,000 yr BP (Van Devender and Spaulding, 1979; Van Devender et al., 1987; Anderson, 1989; Betancourt, 1990; Anderson et al., 1999). This appears to have been enabled by the succession of cold, moist conditions of the late Wisconsin with warmer, moist conditions of the Holocene that included an intensification of the summer monsoon (Anderson, 1989; Thompson et al., 1993). The distributional pattern of Haplotype 3, from southern Arizona to northern Wyoming and from Nevada to Oklahoma, appears consistent with rapid northward movement from areas near the known glacial refugia in southern Arizona and New Mexico.

It is additionally possible that ponderosa pine may have survived in isolated mesic microhabitats further north than southern New Mexico and Arizona, although no fossil data currently support this hypothesis (Anderson, 1989; Rehfeldt, 1999b). The results of our study seem to offer evidence that this was the case. For example, Haplotype 7, which appears to be sister to most or all other ponderosa pine haplotypes, exists only in the eastern Great Basin and northern Colorado Plateau. It is unlikely either to have evolved since the end of the Pleistocene, or to have migrated from a refugium further south. The high topographic diversity in the Great Basin, however, may have allowed the existence of many habitats on mountains throughout the Quaternary (Charlet, 2007), including places where this haplotype may have survived. The same may be the case in the Sheep Range of southern Nevada, where three haplotypes coexist in close proximity; interestingly, ponderosa pine appears as a dominant species in a 10 000-yr-old packrat midden from this area (Van Devender and Spaulding, 1979). Our mtDNA results may suggest that, contrary to the paleobotanical data (Wells, 1983a; Thompson, 1990), ponderosa pine may have existed in the Great Basin prior to the end of the Pleistocene, and may have been a colonization source for other regions (Charlet, 2007).

The distribution of Haplotype 6 may represent additional evidence of a more northerly, as-yet undetected glacial refugium. It exists only from southern Colorado north to Montana, and appears to be intermediate between Haplotypes 7 and 3. The location of this haplotype suggests that it may have existed in one or more Pleistocene refugia north and east of the refugium or refugia which harbored Haplotype 3. One possible location is the High Plains, including northern Texas (Wells, 1970) and western Kansas (Wells, 1983b). The spread of ponderosa pine from this region would likely have been impeded by the crest of the Rocky Mountains, allowing Haplotype 3 to move north along the western slope of the range. The distribution of mtDNA haplotypes in a study of *Pinus flexilis* E. James lead the authors

to conclude that this species had multiple northern refugia, including on the High Plains along the Rocky Mountains, in the Southern Rockies, in the Northern Rockies, and in Utah, in addition to southern New Mexico and in Arizona (Mitton et al., 2000b). Several of these appear to correspond generally with potential refugial areas for ponderosa pine.

Populations of *P. ponderosa* var. *scopulorum* are thought to have exhibited a largely latitudinal post-Pleistocene movement, probably coupled with altitudinal expansion (Anderson, 1989). This hypothesis is confirmed both by the geographic pattern of haplotypes in the region, and by the significant correlation detected between population latitude and population-level haplotypic diversity in the Northern Rocky Mountains race (Table 4). Ponderosa pine appears to have reached north-central Colorado by at least 5,100 yr BP (Betancourt, 1987), southeastern Wyoming by 4,060 yr BP (Wells, 1970), and the secondary contact zone with *P. ponderosa* var. *ponderosa* in western Montana between 1,000 and 5,000 yr BP (Betancourt et al., 1990). The stark division we found here between the *P. ponderosa* var. *ponderosa* and var. *scopulorum* haplotypes is consistent with previous mtDNA work that found a sharp cline in variation within the secondary contact zone between the varieties in Montana, while the cline in cpDNA variation was attenuated by greater gene flow (Latta and Mitton, 1999). The mtDNA cline in this secondary contact zone was less than 10 km wide, with no evidence of intermixing or of a mosaic contact zone between the two varieties (Johansen and Latta, 2003). Movement of seed since secondary contact clearly has been insufficient to homogenize the two historically separate groups (Latta and Mitton, 1999), underscoring the relatively short period they have been in contact.

Understanding the evolutionary history of Haplotypes 2 and 4, meanwhile, presents a challenge. These haplotypes, along with only Haplotype 7, share a deletion of the D minisatellite motif, and are only two mutational steps apart. The facts that Haplotype 4 was found only in two isolated high-elevation areas in southern Nevada and that it co-occurs with Haplotype 2 (Fig. 2) suggest it probably originated from Haplotype 2 as a result of recent mutational events, perhaps as recently as the Holocene (Godbout et al., 2005), or perhaps in a small Pleistocene glacial refugium in the area. The relationship between Haplotypes 2 and 7, however, is less clear. The two are separated by four mutational differences, but share the D motif deletion and occur approximately 130 km apart in the Great Basin. Meanwhile, the deletion of the D motif is the only difference between Haplotypes 2 and 3, and both haplotypes are present together in populations in southern Nevada and southern New Mexico. This pattern, along with the bifurcated distribution of Haplotype 2 (southern California/southern Nevada and southern New Mexico), may suggest that Haplotype 2 moved north from a Pleistocene refugium in the Sierra Nevada of Mexico, possibly along the western and eastern edges of the range and possibly after diverging from Haplotype 3. Alternatively, it may have existed across a broad swath of the southwestern United States and northern Mexico, but may have been extirpated locally in Arizona, or may still exist in this area but has been unsampled in our study. Such an explanation may be more parsimonious than a recent divergence of Haplotype 2 from Haplotype 7, although research with additional DNA markers could potentially help clarify the evolutionary relationship of these haplotypes.

Long-distance dispersal plays a critical role in the migration and population development of *P. ponderosa* (Lesser and Jackson, 2013), which is a species that encompasses many isolated disjunct populations of various sizes (Rehfeldt, 1999b). Long-distance dispersal may not entirely explain the spatial occurrence pattern of Haplotype 2, given that previous research has found that seed dispersal in *P. ponderosa* was generally spatially limited (Oliver and Ryker, 1990; Latta et al., 1998) and that continued low-frequency long-distance dispersal was necessary not only for initial *P. ponderosa* colonization but also to sustain subsequent population growth (Lesser and Jackson, 2013). While *P. ponderosa* seeds are thought to be dispersed mostly by wind and gravity, however, corvid birds, such as Clark's nutcracker (*Nucifraga columbiana* Wilson), are known to feed on and cache *P. ponderosa* seeds (Lorenz et al., 2008), and have been found to disperse whitebark pine (*P. albicaulis*) seeds as far as 30 km (Barringer et al., 2012), with most seed caching occurring within 100 m to 3.5 km of the seed source (Tomback, 2001). Such seed movements are highly likely to have influenced distributional patterns of haplotypes such as 1, 3, 5, and 8 as they moved north following the end of the Pleistocene.

***Implications for taxonomic treatments of* Pinus ponderosa—** Ponderosa pine may best be considered a complex of evolutionary units (Moritz, 1994), but the number and location of those units has been a matter of much debate. Researchers have documented considerable variation across the range of ponderosa pine in morphology (Weidman, 1939; Squillace and Silen, 1962; Wells, 1964; Haller, 1965; La Farge, 1975; Read, 1980), growth (Squillace and Silen, 1962; Wells, 1964; Wright et al., 1969; Read, 1980, 1983; Van Haverbeke, 1986), cold hardiness (Wells, 1964; Read, 1980; Rehfeldt, 1993), disease resistance (Hoff, 1988), monoterpene composition (Smith et al., 1969; Smith, 1977; Sturgeon, 1979), and allozymes (Conkle and Critchfield, 1988; Niebling and Conkle, 1990). This variation has resulted in the division of the species into the widely accepted Pacific (var. *ponderosa*) and Rocky Mountain (var. *scopulorum*) varieties, but has also led some taxonomists to further divide each variety. Rocky Mountain ponderosa pine in the Southwestern United States, for example, has been treated as a species (*Pinus brachyptera* Engelm.) and as a variety (*P. ponderosa* var. *brachyptera* (Engelm.) Lemmon). Similarly, Pacific ponderosa pine in California and southwestern Oregon has been treated as a species (*P. benthamiana* Hartw.), subspecies (*P. ponderosa* subsp. *benthamiana* (Hartw.) Silba) and variety (*P. ponderosa* var. *benthamiana* (Hartw.) Vasey). Additionally, *P. arizonica* Engelm., which generally possesses five-needle fascicles and occurs in scattered populations in southern New Mexico and Arizona and in northern Mexico, has been treated as variety of ponderosa pine (*P. ponderosa* var. *arizonica* (Engelm.) Shaw), but has different enough monoterpene content (Peloquin, 1984), cone and needle morphology (Perry, 1991), and seedling characteristics (Rehfeldt, 1999b) to be considered a distinct species.

Conflicting geographic patterns of variation in several traits, however, have created confusion about the evolutionary history and proper taxonomic treatment of the ponderosa pine complex. For example, genetic distances based on allozyme allele frequencies indicated a close genetic relationship between Pacific Coast and North Plateau ponderosa pines (*P. ponderosa* var. *ponderosa*), and more distant relationships between these races and the ponderosa pines of the Rocky Mountains (*P. ponderosa* var. *scopulorum*) (Niebling and Conkle, 1990). At the same time, xylem resin monoterpene characteristics were indistinguishable between North Plateau (*P. ponderosa* var. *ponderosa*)

and Rocky Mountain (*P. ponderosa* var. *scopulorum*) races (Smith, 1977), while exhibiting dramatic differences between the North Plateau and Pacific Coast races of *P. ponderosa* var. *ponderosa* (Smith, 1977; Sturgeon, 1979). A study of leaf oil terpene composition, meanwhile, indicated that *P. ponderosa* var. *ponderosa* was remarkably uniform, while detecting strong differences between *P. ponderosa* var. *ponderosa* and *P. ponderosa* var. *scopulorum* (Rudloff and Lapp, 1992).

As noted previously, the results of our study are consistent with the commonly proposed separation of ponderosa pine into Pacific and Rocky Mountain varieties, including in the correspondence between haplotype distribution and commonly recognized variety. The only exception appears in Southern California, the location of three populations consisting entirely of Haplotype 2, which is otherwise present only within the range of the previously proposed Rocky Mountain variety. This Haplotype 2 distribution pattern also may be reflected in a sharp transition in monoterpene races between ponderosa pine populations in the Transverse Ranges of Southern California and populations in the Sierra Nevada and Cascade ranges (Smith, 1977). Given those findings, the assignment of ponderosa pine populations in Southern California to the Pacific variety may require additional consideration. Analysis of potential hybridization between *P. ponderosa* and *P. jeffreyi* in this region also deserves further study given that the two species are sympatric there. Controlled crosses have verified that the species have strong barriers to crossing but that they will hybridize (Conkle and Critchfield, 1988), while hybridization was found to transfer a chloroplast from *P. jeffreyi* to a sympatric *P. washoensis* individual (Willyard et al., 2009). It is unclear, however, how hybridization in southern California would result in the presence of a *P. ponderosa* mtDNA haplotype that also exists hundreds of kilometers farther east in locations lacking *P. jeffreyi*, while populations farther north do not possess this haplotype despite containing both species.

Our results, meanwhile, do not offer strong support for further subdivision of the commonly recognized Pacific variety and offer weak support for further subdivision of the commonly recognized Rocky Mountain variety. First, the haplotypes within the varieties do not appear to segregate into the previously proposed northern and southern races for each; for example, Haplotype 1 spans both in the Pacific variety and Haplotype 3 spans both in the Rocky Mountain variety. Haplotype 8, however, is confined to the North Plateau race, Haplotype 5 is mostly associated with the Pacific Coast race, Haplotypes 6 and 7 are found only within the Northern Rocky Mountain race, and Haplotypes 2 and 3 are located only within the Southwestern race. A greater amount of variance in the previously proposed Rocky Mountain variety, however, was attributed to variation among races (27%) than in the previously proposed Pacific variety (16%) (Table 5), suggesting that ponderosa pine in this region may be farther along in the process of evolutionary differentiation.

Finally, our results appear to argue against species status for Washoe pine (*P. washoensis*), a small-coned taxon that is native to three high-elevation locations on the western rim of the Great Basin in northeastern California and northwestern Nevada (Critchfield and Allenbaugh, 1965; Critchfield, 1984). The two Washoe pine populations (#17 and #18) have the same haplotype as nearby populations in the Sierra Nevada. Given the distribution of Haplotype 1, our results appear to support the hypotheses that Washoe pine is closely related to the North Plateau race of ponderosa pine (Critchfield, 1984; Niebling and Conkle, 1990; Rehfeldt, 1999a), that it is not allied with *P. ponderosa* var. *scopulorum* and *P. arizonica* as proposed by Axelrod (1986), and that it is not differentiated enough to be considered a separate species (Brayshaw, 1997; Lauria, 1997; Rehfeldt, 1999a; Gernandt et al., 2009; Willyard et al., 2009). The mtDNA results also do not support varietal status, as proposed by Haller and Vivrette (2011), i.e., *P. ponderosa* var. *washoensis* (H.L. Mason & Stockw.) J.R. Haller and N.J. Vivrette.

Hybridization may be a factor in the morphological differentiation of Washoe pine, particularly considering that the combination of incomplete mating barriers among pines and the opportunity for secondary contact may have allowed infrequent but evolutionarily significant introgression (Willyard et al., 2009). Rare hybridization events followed by selection, meanwhile, can cause the displacement of a species' mtDNA haplotype with that of a sympatric congener (Shaw, 2002). While our mtDNA results provided no evidence of recent introgression into Washoe pine, the results of a chloroplast sequencing study supported the potential for a low level of ongoing introgression from *Pinus jeffreyi* (Willyard et al., 2009).

***Conservation and management implications*—**The results of our study demonstrate the existence of important regional evolutionary differences within *P. ponderosa*, and may reveal phylogeographic processes that generated these differences. This information should assist in the appropriate management decision-making and conservation planning relating to ponderosa pine subordinate taxa and populations in the face of multiple threats, including those associated with climate change (Gitlin et al., 2006; Aitken et al., 2008; Williams et al., 2010).

Phylogenetic analyses involving conspecific populations often reveal multiple lineages that may warrant protection as evolutionarily distinct units (Soltis and Gitzendanner, 1999). Ponderosa pine is a forest tree species in which morphological races and varieties may be in the early stages of speciation (Jaramillo-Correa et al., 2009). Each of the haplotypes we detected may represent an evolutionarily distinct unit that may respond differently to climate change because of differences in adaptation to climate conditions as a result of long-term isolation. Previous work has demonstrated the importance of considering *P. ponderosa* variety in biogeographical modeling and ecological forecasting in the face of climate change (Norris et al., 2006). It may additionally be necessary to assess whether haplotypic subgroups may also respond differentially in response to climate change. Additionally, climate change and a long-term bark beetle outbreak across western North America (Chapman et al., 2012; Meddens et al., 2012), which has been exacerbated by warmer climate conditions (Mitton and Ferrenberg, 2012), may require prioritization of *P. ponderosa* populations for conservation measures, including seed archiving, silvicultural treatments, and prescribed fire. Such efforts should consider the presence of a rare haplotype (such as Haplotypes 2, 4, 9, and 10) and the existence of high haplotypic diversity (such as in populations 7, 8, 22, and 34).

Finally, further range-wide analyses using polymorphic, codominant nuclear markers such as microsatellites (e.g., Boys et al., 2005; Dvorak et al., 2009; Potter et al., 2012) or allozymes (e.g., Gibson and Hamrick, 1991; Jorgensen and Hamrick, 1997; Schmidtling and Hipkins, 1998) may identify areas harboring high levels of genetic variation, offer a better sense of recent population processes such as genetic bottlenecks and recent gene flow, and provide additional data for evaluating the phylogeographic patterns inferred in this analysis.

Conclusions—Across a range-wide sample of 104 *P. ponderosa* populations, we detected 10 size variants in the *nad1* second intron mtDNA minisatellite region. Each haplotype consisted of a distinct pattern of imperfect tandem repeats; aligned repeat motifs allowed for the estimation of evolutionary relationships among haplotypes. Haplotype distributional patterns across the range of *P. ponderosa*, and the evolutionary relationships among the haplotypes, suggest a complex phylogeographic history not revealed by other genetic and morphological data, or by the sparse paleoecological record. The pattern of haplotype distribution and the partitioning of haplotypic diversity are consistent with the separation of ponderosa pine into Pacific and Rocky Mountain varieties. The results, however, do not offer strong support for further subdivision of the commonly recognized Pacific variety and only weak support for further subdivision of the commonly recognized Rocky Mountain variety, and appear to argue against species status for Washoe pine. The results suggest that Pleistocene refugia may have existed in areas of high haplotypic diversity, including the Sierra Nevada mountains, southern Nevada and northwestern California, as well as in the Great Basin, in the southwestern United States/northern Mexico, and on the High Plains. These findings should be useful for the management and conservation of this widespread and important species in the face of threats that include climate change and bark beetle infestations. *P. ponderosa* has a broad environmental and geographic distribution across western North America, so these results offer insights into how biogeography and climate may have affected the modern evolutionary structure of other species in the region.

LITERATURE CITED

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

ANDERSON, R. S. 1989. Development of the southwestern ponderosa pine forests: What do we really know? *In* A. Tecle, W. W. Covington, and R. H. Hamre [eds.], Multiresource management of ponderosa pine forests. USDA Forest Service General Technical Report RM-185, 15-22. Rocky Mountain Forest and Range Experiment Station, Fort Collins, Colorado, USA.

ANDERSON, R. S. 1990. Holocene forest development and paleoclimates within the central Sierra Nevada, California. *Journal of Ecology* 78: 470–489.

ANDERSON, R. S. 1993. A 35,000 year vegetation and climate history from Potato Lake, Mogollon Rim, Arizona. *Quaternary Research* 40: 351–359.

ANDERSON, R. S., J. L. BETANCOURT, J. I. MEAD, R. H. HEVLY, AND D. P. ADAM. 2000. Middle- and late-Wisconsin paleobotanic and paleoclimatic records from the southern Colorado Plateau, USA. *Palaeogeography, Palaeoclimatology, Palaeoecology* 155: 31–57.

ANDERSON, R. S., J. HASBARGEN, P. A. KOEHLER, AND E. J. FEILER. 1999. Late Wisconsin and Holocene subalpine forests of the Markagunt Plateau of Utah, southwestern Colorado Plateau, USA. *Arctic, Antarctic, and Alpine Research* 31: 366–378.

AXELROD, D. I. 1986. Cenozoic history of some western American pines. *Annals of the Missouri Botanical Garden* 73: 565–641.

BAKER, R. G. 1983. Holocene vegetational history of the western United States. *In* H. E. Wright Jr. [ed.], Late-Quaternary environments of the United States, vol. 2, 109-127. University of Minnesota Press, Minneapolis, Minnesota, USA.

BARNOSKY, C. W., P. M. ANDERSON, AND P. J. BARTLEIN. 1987. The northwestern U.S. during deglaciation: Vegetational history and paleoclimatic implications. *In* W. F. Ruddiman and H. E. Wright Jr. [eds.], North America and adjacent oceans during the last deglaciation, vol. K-3, The geology of North America, 289-321. Geological Society of America, Boulder, Colorado, USA.

BARRINGER, L. E., D. F. TOMBACK, M. B. WUNDER, AND S. T. MCKINNEY. 2012. Whitebark pine stand condition, tree abundance, and cone production as predictors of visitation by Clark's nutcracker. *PLoS ONE* 7(5): e37663.

BERARD, S., F. NICOLAS, J. BUARD, O. GASCUEL, AND E. RIVALS. 2006. A fast and specific alignment method for minisatellite maps. *Evolutionary Bioinformatics* 2: 303–320.

BERARD, S., AND E. RIVALS. 2003. Comparison of minisatellites. *Journal of Computational Biology* 10: 357–372.

BETANCOURT, J. L. 1987. Paleoecology of pinyon-juniper woodlands: Summary. *In* R. L. Everett [ed.], Proceedings of the Pinyon-Juniper Conference in Reno, Nevada. USDA Forest Service General Technical Report 215, 129-139. Intermountain Research Station, Reno, Nevada, USA.

BETANCOURT, J. L. 1990. Late Quaternary biogeography of the Colorado Plateau. *In* J. L. Betancourt, T. R. Van Devender, and P. S. Martin [eds.], Packrat middens: The last 40,000 years of biotic change, 259-292. University of Arizona Press, Tucson, Arizona, USA.

BETANCOURT, J. L., T. R. VAN DEVENDER, AND P. S. MARTIN. 1990. Packrat middens: The last 40,000 years of biotic change, 447. University of Arizona Press, Tucson, Arizona, USA.

BIRKY, C. W., P. FUERST, AND T. MARUYAMA. 1989. Organelle gene diversity under migration, mutation, and drift: Equilibrium expectations, approach to equilibrium, effets of heteroplasmic cells, and comparison to nuclear genes. *Genetics* 121: 613–627.

BONHOMME, F., E. RIVALS, A. ORTH, G. R. GRANT, A. J. JEFFREYS, AND P. R. J. BOIS. 2007. Species-wide distribution of highly polymorphic minisatellite markers suggests past and present genetic exchanges among house mouse subspecies. *Genome Biology* 8(5): R80.

BOYS, J., M. CHERRY, AND S. DAYANANDAN. 2005. Microsatellite analysis reveals genetically distinct populations of red pine (*Pinus resinosa* Pinaceae). *American Journal of Botany* 92: 833–841.

BRAYSHAW, T. C. 1997. Washoe and ponderosa pines on Promontory Hill near Merritt, B.C., Canada. *Annalen des Naturhistorischen Museums in Wien* 99B: 673–680.

CALSBEEK, R., J. N. THOMPSON, AND J. E. RICHARDSON. 2003. Patterns of molecular evolution and diversification in a biodiversity hotspot: The California floristic province. *Molecular Ecology* 12: 1021–1029.

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

CASSEL, E. J., S. A. GRAHAM, C. P. CHAMBERLAIN, AND C. D. HENRY. 2012b. Early Cenozoic topography, morphology, and tectonics of the northern Sierra Nevada and western basin and range. *Geosphere* 8: 229–249.

CASSEL, E. J., M. GROVE, AND S. A. GRAHAM. 2012a. Eocene draining evolution and erosion of the Sierra Nevada batholith across northern California and Nevada. *American Journal of Science* 312: 117–144.

CASSENS, I., P. MARDULYN, AND M. C. MILINKOVITCH. 2005. Evaluating intraspecific "Network" construction methods using simulated sequence data: Do existing algorithms outperform the global maximum parsimony approach? *Systematic Biology* 54: 363–372.

CHAMBERLAIN, C. P., H. T. MIX, A. MULCH, M. T. HREN, M. L. KENT-CORSON, S. J. DAVIS, T. W. HORTON, ET AL. 2012. The Cenozoic climatic and topographic evolution of the western North American cordillera. *American Journal of Science* 312: 213–262.

CHAMBERLAIN, C. P., AND M. A. POAGE. 2000. Reconstructing the paleotopography of mountain belts from the isotopic composition of authigenic minerals. *Geology* 28: 115–118.

CHAPMAN, T. B., T. T. VEBLEN, AND T. SCHOENNAGEL. 2012. Spatiotemporal patterns of mountain pine beetle activity in the southern Rocky Mountains. *Ecology* 93: 2175–2185.

CHARLET, D. A. 2007. Distribution patterns of Great Basin conifers: Implications of extinction and immigration. *Aliso* 24: 31–61.

COLE, K. 1983. Late Pleistocene vegetation of Kings Canyon, Sierra Nevada, California. *Quaternary Research* 19: 117–129.

COLE, K. L. 1981. Late Quaternary developments in the eastern Grand Canyon: Vegetational gradients over the last 25,000 years. Ph.D. dissertation, University of Arizona, Tucson, Arizona, USA.

COLGAN, J. P., AND C. D. HENRY. 2009. Rapid middle Miocene collapse of the Mesozoic orogenic plateau in north-central Nevada. *International Geology Review* 51: 920–961.

CONKLE, M. T., AND W. B. CRITCHFIELD. 1988. Genetic variation and hybridization of ponderosa pine. *In* D. M. Baumgartner and J. E. Lotan [eds.], Ponderosa pine: The species and its management, 27-43. Washington State University Cooperative Extension, Pullman, Washington, USA.

CRITCHFIELD, W. B. 1984. Crossability and relationships of Washoe pine. *Madroño* 31: 144–170.

CRITCHFIELD, W. B., AND G. L. ALLENBAUGH. 1965. Washoe pine on the Bald Mountain Range, California. *Madroño* 18: 63–64.

CRITCHFIELD, W. B., AND E. L. LITTLE. 1966. Geographic distribution of the pines of the world. USDA Forest Service Miscellaneous Publication 991, Washington, District of Columbia, USA.

DEMESURE, B., N. SODZI, AND R. J. PETIT. 1995. A set of universal primers for amplification of polymorphic noncoding regions of mitochondrial and chloroplast DNA in plants. *Molecular Ecology* 4: 129–131.

DESPER, R., AND O. GASCUEL. 2002. Fast and accurate phylogeny reconstruction algorithms based on the minimum-evolution principle. *Journal of Computational Biology* 9: 687–705.

DONG, J., AND D. B. WAGNER. 1993. Taxonomic and population differentiation of mitochondrial diversity in *Pinus banksiana* and *Pinus contorta. Theoretical and Applied Genetics* 86: 573–578.

DONG, J. S., AND D. B. WAGNER. 1994. Paternally inherited chloroplast polymorphism in *Pinus*: Estimation of diversity and population subdivision, and tests of disequilibrium with a maternally inherited mitochondrial polymorphism. *Genetics* 136: 1187–1194.

DVORAK, W. S., K. M. POTTER, V. D. HIPKINS, AND G. R. HODGE. 2009. Genetic diversity and gene exchange in *Pinus oocarpa*, a Mesoamerican pine with resistance to the pitch canker fungus (*Fusarium circinatum*). *International Journal of Plant Sciences* 170: 609–626.

ENNOS, R. A. 1994. Estimating the relative rates of pollen and seed migration among plant populations. *Heredity* 72: 250–259.

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

FARJON, A. 1984. Pines: Drawings and descriptions of the genus *Pinus*. E.J. Brill, Leiden, Netherlands.

FURNIER, G. R., AND W. T. ADAMS. 1986. Geographic patterns of allozyme variation in Jeffrey pine. *American Journal of Botany* 73: 1009–1015.

GASCUEL, O., AND M. STEEL. 2006. Neighbor-joining revealed. *Molecular Biology and Evolution* 23: 1997–2000.

GERNANDT, D. S., S. HERNANDEZ-LEON, E. SALGADO-HERNANDEZ, AND J. A. P. DE LA ROSA. 2009. Phylogenetic relationships of *Pinus* subsection *Ponderosae* inferred from rapidly evolving cpDNA regions. *Systematic Botany* 34: 481–491.

GERSON, E. A., R. G. KELSEY, AND J. B. ST CLAIR. 2009. Genetic variation of piperidine alkaloids in *Pinus ponderosa*: A common garden study. *Annals of Botany* 103: 447–457.

GIBSON, J. P., AND J. L. HAMRICK. 1991. Genetic diversity and structure in *Pinus pungens* (Table Mountain pine) populations. *Canadian Journal of Forest Research-Revue Canadienne de Recherche Forestiere* 21: 635–642.

GITLIN, A. R., C. M. STHULTZ, M. A. BOWKER, S. STUMPF, K. L. PAXTON, K. KENNEDY, A. MUÑOZ, ET AL. 2006. Mortality gradients within and among dominant plant populations as barometers of ecosystem change during extreme drought. *Conservation Biology* 20: 1477–1486.

GODBOUT, J., A. FAZEKAS, C. H. NEWTON, F. C. YEH, AND J. BOUSQUET. 2008. Glacial vicariance in the Pacific Northwest: Evidence from a lodgepole pine mitochondrial DNA minisatellite for multiple genetically distinct and widely separated refugia. *Molecular Ecology* 17: 2463–2475.

GODBOUT, J., J. P. JARAMILLO-CORREA, J. BEAULIEU, AND J. BOUSQUET. 2005. A mitochondrial DNA minisatellite reveals the postglacial history of jack pine (*Pinus banksiana*), a broad-range North American conifer. *Molecular Ecology* 14: 3497–3512.

GUGGER, P. F., S. SUGITA, AND J. CAVENDER-BARES. 2010. Phylogeography of Douglas-fir based on mitochondrial and chloroplast DNA sequences: Testing hypotheses from the fossil record. *Molecular Ecology* 19: 1877–1897.

HALLER, J. R. 1965. The role of 2-needle fascicles in the adaptation and evolution of ponderosa pine. *Brittonia* 17: 354–382.

HALLER, J. R., AND N. J. VIVRETTE. 2011. Ponderosa pine revisited. *Aliso* 29: 53–57.

HENRY, C. D. 2009. Uplift of the Sierra Nevada, California. *Geology* 37: 575–576.

HOFF, R. J. 1988. Susceptibility of ponderosa pine to the needle case fungus *Lophodermium baculiferum*. USDA Forest Service Research Paper INT-386., Intermountain Research Station, Ogden, Utah, USA.

HUFF, D. R. 1997. RAPD characterization of heterogeneous perennial ryegrass cultivars. *Crop Science* 37: 557–564.

HUFF, D. R., R. PEAKALL, AND P. E. SMOUSE. 1993. RAPD variation within and among natural populations of outcrossing buffalograss (*Buchloe dactyloides* (Nutt.) Engelm.). *Theoretical and Applied Genetics* 86: 927–934.

JARAMILLO-CORREA, J. P., J. BEAULIEU, D. P. KHASA, AND J. BOUSQUET. 2009. Inferring the past from the present phylogeographic structure of North American forest trees: Seeing the forest for the genes. *Canadian Journal of Forest Research-Revue Canadienne de Recherche Forestiere* 39: 286–307.

JOHANSEN, A. D., AND R. G. LATTA. 2003. Mitochondrial haplotype distribution, seed dispersal and patterns of postglacial expansion of ponderosa pine. *Molecular Ecology* 12: 293–298.

JORGENSEN, S. M., AND J. L. HAMRICK. 1997. Biogeography and population genetics of whitebark pine, *Pinus albicaulis. Canadian Journal of Forest Research-Revue Canadienne de Recherche Forestiere* 27: 1574–1585.

KITCHEN, S. G. 2010. Historic fire regimes of eastern Great Basin (USA) mountains reconstructed from tree rings. Ph.D. dissertation, Brigham Young University, Provo, Utah, USA.

LA FARGE, T. 1975. Genetic differences in stem form of ponderosa pine grown in Michigan. *Silvae Genetica* 23: 211–213.

LASCOUX, M., A. E. PALME, R. CHEDDADI, AND R. G. LATTA. 2004. Impact of Ice Ages on the genetic structure of trees and shrubs. *Philosophical Transactions of the Royal Society of London. B, Biological Sciences* 359: 197–207.

LATTA, R. G., Y. B. LINHART, D. FLECK, AND M. ELLIOT. 1998. Direct and indirect estimates of seed versus pollen movement within a population of ponderosa pine. *Evolution; International Journal of Organic Evolution* 52: 61–67.

LATTA, R. G., AND J. B. MITTON. 1999. Historical separation and present gene flow through a zone of secondary contact in ponderosa pine. *Evolution; International Journal of Organic Evolution* 53: 769–776.

LAURIA, F. 1997. The taxonomic status of *Pinus washoensis* H. Mason & Stockw. (Pinaceae). *Annalen des Naturhistorischen Museums in Wien* 99: 655–671.

LESSER, M. R., AND S. T. JACKSON. 2013. Contributions of long-distance dispersal to population growth in colonising Pinus ponderosa populations. *Ecology Letters* 16: 380–389.

LI, P., AND W. T. ADAMS. 1989. Range-wide patterns of allozyme variation in Douglas-fir (*Pseudotsuga menziesii*). *Canadian Journal of Forest Research-Revue Canadienne de Recherche Forestiere* 19: 149–161.

LITTLE, E. L. 1971. Atlas of United States Trees, vol. 1. Conifers and important hardwoods. USDA Forest Service Miscellaneous Publication 1146. Washington, District of Columbia, USA.

LORENZ, T. J., C. AUBRY, AND R. SHOAL. 2008. A review of the literature on seed fate in whitebark pine and the life history traits of Clark's nutcracker and pine squirrels. USDA Forest Service General Technical Report PNW-GTR 742. Pacific Northwest Research Station, Portland, Oregon, USA.

MACDONALD, G. M., L. C. CWYNAR, AND C. WHITLOCK. 1998. The late Quaternary dynamics of pines in northern North America. *In* D. M.

Richardson [ed.], Ecology and biogeography of *Pinus*, 122-136. Cambridge University Press, Cambridge, UK.

MCPHILLIPS, D., AND M. T. BRANDON. 2012. Topographic evolution of the Sierra Nevada measured directly by inversion of low-temperature thermochronology. *American Journal of Science* 312: 90–116.

MEDDENS, A. J. H., J. A. HICKE, AND C. A. FERGUSON. 2012. Spatiotemporal patterns of observed bark beetle-caused tree mortality in British Columbia and the western United States. *Ecological Applications* 22: 1876–1891.

MIHAESCU, R., D. LEVY, AND L. PACHTER. 2009. Why neighbor-joining works. *Algorithmica* 54: 1–24.

MINNICH, R. A. 2007. Climate, paleoclimate, and paleovegetation. *In* M. G. Barbour, T. Keeler-Wolf, and A. A. Schoenherr [eds.], Terrestrial vegetation of California, 3rd ed., 43-70. University of California Press, Berkeley, California, USA.

MITTON, J. B., AND S. M. FERRENBERG. 2012. Mountain pine beetle develops an unprecedented summer generation in response to climate warming. *American Naturalist* 179: E163–E171.

MITTON, J. B., B. R. KREISER, AND R. G. LATTA. 2000b. Glacial refugia of limber pine (*Pinus flexilis* James) inferred from the population structure of mitochondrial DNA. *Molecular Ecology* 9: 91–97.

MITTON, J. B., B. R. KREISER, AND G. E. REHFELDT. 2000a. Primers designed to amplify a mitochondrial *nad1* intron in ponderosa pine, *Pinus ponderosa*, limber pine, *P. flexilis*, and Scots pine, *P. sylvestris. Theoretical and Applied Genetics* 101: 1269–1272.

MORITZ, C. 1994. Defining evolutionarily significant units for conservation. *Trends in Ecology & Evolution* 9: 373–375.

NEALE, D. B., AND R. R. SEDEROFF. 1989. Paternal inheritance of chloroplast DNA and maternal inheritance of mitochondrial DNA in loblolly pine. *Theoretical and Applied Genetics* 77: 212–216.

NEALE, D. B., N. C. WHEELER, AND R. W. ALLARD. 1986. Paternal inheritance of chloroplast DNA in Douglas-fir. *Canadian Journal of Forest Research-Revue Canadienne de Recherche Forestiere* 16: 1152–1154.

NEI, M. 1987. Molecular evolutionary genetics. Columbia University Press, New York, New York, USA.

NICHOLAS, K. B., H. B. J. NICHOLAS, AND D. W. DEERFIELD. 1997. GeneDoc: Analysis and visualization of genetic variation. *EMBnet.news* 4: 1-4.

NIEBLING, C. R., AND M. T. CONKLE. 1990. Diversity of Washoe pine and comparisons with allozymes of ponderosa pine races. *Canadian Journal of Forest Research-Revue Canadienne de Recherche Forestiere* 20: 298–308.

NORRIS, J. R., S. T. JACKSON, AND J. L. BETANCOURT. 2006. Classification tree and minimum-volume ellipsoid analyses of the distribution of ponderosa pine in the western USA. *Journal of Biogeography* 33: 342–360.

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

OLIVER, W. W., AND R. A. RYKER. 1990. Ponderosa pine. *In* R. M. Burns, and B. H. Honkala [eds.], Silvics of North America: 1. Conifers, vol. 1, USDA Forest Service Agriculture Handbook 654. Washington, District of Columbia, USA.

PARADIS, E., J. CLAUDE, AND K. STRIMMER. 2004. APE: Analyses of phylogenetics and evolution in R language. *Bioinformatics (Oxford, England)* 20: 289–290.

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

PELOQUIN, R. L. 1984. The identification of three-species hybrids in the ponderosa pine complex. *Southwestern Naturalist* 29: 115–122.

PERRY, J. P. 1991. The pines of Mexico and Central America. Timber Press, Portland, Oregon, USA.

PETIT, R. J., A. KREMER, AND D. B. WAGNER. 1993. Finite island model for organelle and nuclear genes in plants. *Heredity* 71: 630–641.

PLEINES, T., S. S. JAKOB, AND F. R. BLATTNER. 2009. Application of noncoding DNA regions in intraspecific analyses. *Plant Systematics and Evolution* 282: 281–294.

PORTER, S. C. 1989. Some geological implications of average Quaternary glacial conditions. *Quaternary Research* 32: 245–261.

POTTER, K. M., R. M. JETTON, W. S. DVORAK, V. D. HIPKINS, R. RHEA, AND W. A. WHITTIER. 2012. Widespread inbreeding and unexpected geographic patterns of genetic variation in eastern hemlock (*Tsuga canadensis*), an imperiled North American conifer. *Conservation Genetics* 13: 475–498.

READ, R. A. 1980. Genetic variation in seedling progeny of ponderosa pine provenances. Forest Science, Monograph 23 (Supplement to vol. 26, number 4, 1 December 1980). Society of American Foresters, Washington, District of Columbia, USA.

READ, R. A. 1983. Ten-year performance of ponderosa pine provenances in the Great Plains of North America. USDA Forest Service Research Paper RM-250, Rocky Mountain Forest and Range Experiment Station, Fort Collins, Colorado, USA.

REHFELDT, G. E. 1993. Genetic variation in the Ponderosae of the Southwest. *American Journal of Botany* 80: 330–343.

REHFELDT, G. E. 1999a. Systematics and genetic structure of Washoe pine: Applications in conservation genetics. *Silvae Genetica* 48: 167–173.

REHFELDT, G. E. 1999b. Systematics and genetic structure of Ponderosae taxa (Pinaceae) inhabiting the mountain islands of the Southwest. *American Journal of Botany* 86: 741–752.

RICHARDSON, B. A., J. BRUNSFELD, AND N. B. KLOPFENSTEIN. 2002. 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.

RUDLOFF, E. V., AND M. S. LAPP. 1992. Chemosystematic studies in the genus *Pinus*. VII. The leaf oil terpene composition of ponderosa pine, *Pinus ponderosa. Canadian Journal of Botany* 70: 374–378.

SAITOU, N., AND M. NEI. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4: 406–425.

SALZBURGER, W., G. B. EWING, AND A. VON HAESELER. 2011. The performance of phylogenetic algorithms in estimating haplotype genealogies with migration. *Molecular Ecology* 20: 1952–1963.

SAS Institute. 2008. The SAS system for Windows, Version 9.2. SAS Institute, Cary, North Carolina, USA.

SCHMIDTLING, R. C., AND V. D. HIPKINS. 1998. Genetic diversity in longleaf pine (*Pinus palustris*): Influence of historical and prehistorical events. *Canadian Journal of Forest Research-Revue Canadienne de Recherche Forestiere* 28: 1135–1145.

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

SHAW, K. L. 2002. Conflict between nuclear and mitochondrial DNA phylogenies of a recent species radiation: What mtDNA reveals and conceals about modes of speciation in Hawaiian crickets. *Proceedings of the National Academy of Sciences, USA* 99: 16122–16127.

SINCLAIR, W. T., J. D. MORMAN, AND R. A. ENNOS. 1999. The postglacial history of Scots pine (*Pinus sylvestris* L.) in western Europe: Evidence from mitochondrial DNA variation. *Molecular Ecology* 8: 83–88.

SMITH, F. A., AND J. L. BETANCOURT. 1998. Response of bushy-tailed woodrats (*Neotoma cinerea*) to late Quaternary climatic change in the Colorado Plateau. *Quaternary Research* 50: 1–11.

SMITH, R. H. 1977. Monoterpenes of ponderosa pine xylem resin in western United States. USDA Forest Service Technical Bulletin 1532. Washington, District of Columbia, USA.

SMITH, R. H., R. L. PELOQUIN, AND P. C. PASSOF. 1969. Local and regional variation in the monoterpenes of ponderosa pine wood oleoresin. USDA Forest Service Research Paper PSW- 56, Pacific Southwest Forest and Range Experiment Station, Berkeley, California, USA.

SOLTIS, P. S., AND M. A. GITZENDANNER. 1999. Molecular systematics and the conservation of rare species. *Conservation Biology* 13: 471–483.

SPAULDING, W. G. 1984. The last glacial-interglacial climatic cycle: Its effects on woodlands and forests in the American West. *In* R.M. Lanner [comp.], Proceedings of the 8th North American Forest Biology Workshop: Logan, Utah, July 30-August 1, 1984, 42-69. Department of Forest Resources, Utah State University, Logan, Utah, USA.

SPAULDING, W. G., E. B. LEOPOLD, AND T. R. VAN DEVENDER. 1983. Late Wisconsin paleoecology of the American Southwest. *In* H. E. J. Wright [ed.], Late-Quaternary environments of the United States, volume 1, 259-293. University of Minnesota Press, Minneapolis, Minnesota, USA.

SPELLMAN, G. M., AND J. KLICKA. 2006. Testing hypotheses of Pleistocene population history using coalescent simulations: Phylogeography of the pygmy nuthatch (*Sitta pygmaea*). *Proceedings of the Royal Society of London, B, Biological Sciences* 273: 3057–3063.

SQUILLACE, A. E., AND R. R. SILEN. 1962. Racial variation in ponderosa pine. *Forest Science Monograph* 2: 1–27.

STRAUSS, S. H., D. B. NEALE, AND D. B. WAGNER. 1989. Genetics of the chloroplast in conifers: Biotechnology research reveals some surprises. *Journal of Forestry* 87: 11–17.

STURGEON, K. B. 1979. Monoterpene variation in ponderosa pine xylem resin related to western pine beetle predation. *Evolution; International Journal of Organic Evolution* 33: 803–814.

THOMPSON, R. S. 1990. Late Quaternary vegetation and climate in the Great Basin. *In* J. L. Betancourt, T. R. Van Devender, and P. S. Martin [eds.], Packrat middens: The last 40,000 years of biotic change, 200-239. University of Arizona Press, Tucson, Arizona, USA.

THOMPSON, R. S., C. WHITLOCK, P. J. BARTLEIN, S. P. HARRISON, AND W. G. SPAULDING. 1993. Climatic changes in the western United States since 18,000 yr B.P. *In* H. E. Wright Jr., J. E. Kutzback, T. Webb, W. F. Ruddiman, F. A. Street-Perrott, and P. J. Bartlein [eds.], Global climates since the last glacial maximum, 468-513. University of Minnesota Press, Minneapolis, Minnesota, USA.

TOMBACK, D. F. 2001. Clark's nutcracker: Agent of regneration. *In* D. F. Tomback, S. F. Arno, and R. E. Keane [eds.], Whitebark pine communities: Ecology and restoration, 89-104. Island Press, Washington, District of Columbia, USA.

UNRUH, J. R. 1991. The uplift of the Sierra Nevada and implications for late Cenozoic epeirogeny in the Western Cordillera. *Geological Society of America Bulletin* 103: 1395–1404.

VAN DEVENDER, T. R. 1990. Late Quaternary vegetation and climate of the Chihuahuan Desert, United States and Mexico. *In* J. L. Betancourt, T. R. Van Devender, and P. S. Martin [eds.], Packrat middens: The last 40,000 years of biotic change, 104-132. University of Arizona Press, Tucson, Arizona, USA.

VAN DEVENDER, T. R., J. L. BETANCOURT, AND M. WIMBERLY. 1984. Biogeographic implications of a packrat midden sequence from the Sacramento Mountains, south-central New Mexico. *Quaternary Research* 22: 344–360.

VAN DEVENDER, T. R., AND W. G. SPAULDING. 1979. Development of vegetation and climate in the southwestern United States. *Science* 204: 701–710.

VAN DEVENDER, T. R., R. S. THOMPSON, AND J. L. BETANCOURT. 1987. Vegetation history of the deserts of southwestern North America: The nature and timing of the Late Wisconsin-Holocene transition. *In* W. F. Ruddiman and H. E. Wright Jr. [eds.], North America and adjacent oceans during the last deglaciation, vol. K-3, The geology of North America, 323-352. Geological Society of America, Boulder, Colorado, USA.

VAN HAVERBEKE, D. F. 1986. Genetic variation in ponderosa pine: A 15-year test of provenances in the Great Plains. USDA Forest Service Research Paper RM-265, Rocky Mountain Forest and Range Experiment Station, Fort Collins, Colorado, USA.

WAGNER, D. B., G. R. FURNIER, M. A. SAGHAIMAROOF, S. M. WILLIAMS, B. P. DANCIK, AND R. W. ALLARD. 1987. Chloroplast DNA polymorphisms in lodgepole and jack pines and their hybrids. *Proceedings of the National Academy of Sciences, USA* 84: 2097–2100.

WAKABAYASHI, J., AND T. L. SAWYER. 2001. Stream incision, tectonics, uplift, and evolution of topography of the Sierra Nevada, California. *The Journal of Geology* 109: 539–562.

WANG, C.-W. 1977. Genetics of ponderosa pine. USDA Forest Service Research Paper WO-34. Washington, District of Columbia, USA.

WEIDMAN, R. H. 1939. Evidences of racial influence in a 25-year test of ponderosa pine. *Journal of Agricultural Research* 59: 855-887.

WELLS, O. O. 1964. Geographic variation in ponderosa pine: I. The ecotypes and their distribution. *Silvae Genetica* 13: 89–103.

WELLS, P. V. 1970. Postglacial vegetational history of the Great Plains. *Science* 167: 1574–1582.

WELLS, P. V. 1983a. Paleobiogeography of montane islands in the Great Basin since the last glaciopluvial. *Ecological Monographs* 53: 341–382.

WELLS, P. V. 1983b. Late Quaternary vegetation of the Great Plains. *Transactions of the Nebraska Academy of Sciences* 11: 83–89.

WILLIAMS, A. P., C. D. ALLEN, C. I. MILIAR, T. W. SWETNAM, J. MICHAELSEN, C. J. STILL, AND S. W. LEAVITT. 2010. Forest responses to increasing aridity and warmth in the southwestern United States. *Proceedings of the National Academy of Sciences, USA* 107: 21289–21294.

WILLYARD, A., R. CRONN, AND A. LISTON. 2009. Reticulate evolution and incomplete lineage sorting among the ponderosa pines. *Molecular Phylogenetics and Evolution* 52: 498–511.

WOOLLEY, S. M., D. POSADA, AND K. A. CRANDALL. 2008. A comparison of phylogenetic network methods using computer simulation. *PLoS ONE* 3(4): e1913.

WRIGHT, J. W., W. A. LEMMIEN, AND J. N. BRIGHT. 1969. Early growth of ponderosa pine ecotypes in Michigan. *Forest Science* 15: 121–129.