Molecular Genetic Variation in Whitebark Pine (*Pinus albicaulis* Engelm.) in the Inland West

Paper

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Abstract—Levels of genetic variation within and among 163 individual-tree collections and one bulk lot of whitebark pine were estimated using isozymes, mitochondrial DNA and chloroplast DNA; 79 of the samples are also part of a common garden study evaluating survival, rust resistance, late winter cold hardiness, and early height-growth. Within the species, 100 percent of the isozyme loci are polymorphic, with the number of alleles per locus (Na) equal to 4.0. Genetic diversity is high (H_e = 0.271) relative to other conifers in the same forest cover type and is comparable to quaking aspen (*Populus tremuloides* Michx) and limber pine (*Pinus flexilis* James), two of the most geographically widespread tree species in North America. Fixation values indicate general random mating with no marked excess of heterozygosity or inbreeding. Poor genetic differentiation among zones (F_{ST} = 0.026), low F_{IS} (-0.016) and F_{IT} (0.011) values, and a high number of migrants (N_m = 9.354) also indicate a lack of inbreeding. The oldest known whitebark pine specimen on the Sawtooth National Forest is homozygous for 13 loci (12 for common alleles and one for a rare allele). Of the 164 samples grouped into 117 collection sites, 108 of the *nad5a* intron of the mitochondrial genome contained haplotype 1 present in Idaho, Montana, eastern Washington, and Wyoming, while nine contained haplotype 2 from eastern California and Nevada. This mitochondrial marker, along with high pairwise F_{ST} values, underscores the uniqueness of the Nevada zone. High levels of diversity (H_e = 0.481, Na = 4.2) measured by three, chloroplast simple sequence repeat (SSR) markers indicate the Bitterroots-Idaho Plateau zone has the largest amount of diversity, while the Selkirk-Cabinet zone has the lowest diversity among zones. Similar relationships occur among the Selkirk-Cabinets, Clark Fork-Lolo Pass, and Missions-Glacier Park zones as a group and the Bitterroots-Idaho Plateau and Central Montana zones as another distinct group. Until further sources can be evaluated south of 44.5° N latitude for key adaptive traits, a conservative approach maintains the Bitterroots-Idaho Plateau and Central Montana groups as distinct zones. The four adaptive traits from the common garden study, isozyme data and three chloroplast SSR markers support the Greater Yellowstone-Grand Teton zone remaining a distinct zone. Taken collectively there is sufficient genetic diversity and genetic variation to support the continuation of a rust resistance screening and genetic restoration program for this species.

Introduction

Evolutionary forces of gene mutation, gene flow, random drift, and selection shape the genetic structure of a species. Examples of contemporary forces shaping whitebark pine include wildland fire, fire suppression and exclusion, blister rust (*Cronartium ribicola* A. Dietr.), and mountain pine beetle (*Dendroctonus ponderosae* Hopkins). Uncontrolled wildfire can kill young whitebark pine regeneration or trees of cone-bearing age, which will limit the food supply for dependent wildlife and cause loss of future seed sources for restoration purposes. Wildfires during the 2000 fire season burned 929.2 thousand hectares on USDA National Forest System lands in Idaho and Montana. Much of the fire occurred in higher elevation populations resulting in the reduction of both diseased and healthy whitebark pine trees (Mahalovich and Dickerson 2004). Wildfire aids in the preparation of a seedbed for natural regeneration. Fire suppression and the policy of fire exclusion has reduced the role of fire in regeneration of pure whitebark pine stands and has allowed successional replacement in mixed-conifer stands to subalpine fir (*Abies lasiocarpa* (Hook.) Nutt.), lodgepole pine (*Pinus contorta* Douglas ex Louden) and Engelmann spruce (*Picea engelmannii* Parry ex Engelm.). The frequency and magnitude of these evolutionary forces has the potential to reduce or eliminate available seed sources and advanced reproduction. Even with the recent introduction of a non-native rust pathogen, blister rust resistance is present in the Northern Rockies (Mahalovich and others 2006). Local adaptation is sustainable relative to blister rust resistance, owing to whitebark pine having a generalist adaptive strategy, where the largest proportion of genetic variation is within seed sources (Mahalovich submitted).

A successful genetic restoration program depends on understanding a species' genetic structure. Obtaining basic genetic information facilitates the development of seed transfer guidelines, operational cone collection protocols, selective breeding and gene conservation strategies, and priority setting of high risk areas in need of management intervention through site-specific prescriptions. The first whitebark pine cone collections began in 1991, with a combination of 26 individual-tree and bulked collections in Montana on the Bitterroot, Custer, Gallatin, and Lewis and Clark National Forests. By 1998, the importance of conserving whitebark pine became critical due to the combination of blister rust infection, mountain pine beetle, wildland fire in high elevation ecosystems, and emerging concerns over seed viability in long-term storage. Proactive restoration efforts with a genetic component began in earnest in 1999 (Mahalovich and Dickerson 2004).

Successful tree planting has improved since the earliest efforts in 1991 due to the selection of planting sites best suited for whitebark pine, the application of appropriate seed transfer guidelines, and the development of blister rust resistant

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planting stock. The presence of genetic differentiation in whitebark pine due to directional selection and adaptation to local environments has managerial implications with respect to the control of seed movement in the form of zoning or expert systems.

Seed zones are geographic subdivisions within a region encompassing areas of similar environmental conditions with possible altitudinal limits within zones. When boundaries among subdivisions do not reflect patterns of genetic variation in traits inferring adaptive value, these subdivisions are referred to as provisional seed zones. Provisional seed zones in the Inland West were delineated based on early rust screening trials, the orientation of mountain ranges, and blister rust hazard ratings over large geographic areas (Mahalovich 2000, Mahalovich and Dickerson 2004). Hazard rating systems measure the susceptibility of forested areas to a particular disease by evaluating its impact in a specific host species (for example, percent of trees from an area infected with blister rust and the average number of cankers per tree from that location). These zonal boundaries were conservative in scope (Mahalovich and Dickerson 2004) until genetic data for adaptive traits became available (Mahalovich submitted).

While common garden studies provide essential information regarding the genetic structure in adaptive traits, population genetic studies are also necessary to define genetic structure, genetic diversity, and levels of inbreeding in neutral markers (for example, isozymes or allozymes, mitochondrial DNA (mtDNA), and chloroplast DNA (cpDNA) (Mitton 1995)). Both types of studies share a critical role in determining the effectiveness of a genetic restoration program. Population genetics research using allozymes show little population structure in a range-wide study (F_{ST} = 0.034, Jørgensen and Hamrick 1997), and moderate differentiation in regional (F_{ST} = 0.025, Bruerderle and others 1998) or isolated population studies (F_{ST} = 0.088, Yandell 1993). Fine-scale genetic structure of whitebark pine in the eastern Sierra Nevada Range of California also reveal negligible genetic differentiation among three watersheds (F_{ST} = 0.004) and strong family structure (growth form) due to the seed-caching behavior of Clark's nutcracker (*Nucifraga columbiana* Wilson) (Rogers and others 1999).

Mitochondrial markers track seed movement since mtDNA is maternally inherited in the Pinaceae. Richardson and others (2002) identified variation in the *nad5a* intron of the mitochondrial genome in whitebark pine. This variation was found to occur in a sequence recognized by the restriction endonuclease *Mse*I, resulting in two haplotypes at that locus. After amplifying the *nad5a* intron, one haplotype is cut by *Mse*I, and can be identified by the presence of two bands, while the other haplotype does not contain a restriction site and produces a single band. Evaluation of seed movement in this broader sampling of whitebark pine seed lots, as compared to Richardson and others (2002) is highly desirable.

Isozyme analyses are well established and a cost-effective approach for estimating measures of genetic structure (amount and pattern of variation among and within seed zones), genetic diversity (heterozygosity) and mating systems (outcrossing rate) (USDA Forest Service 2003). These quantitative measures are also comparable to upwards of 3,000 whitebark pine samples submitted to the National Genetic Laboratory in Placerville, CA (USDA Forest Service 2009).

Chloroplast markers track pollen movement since cpDNA is paternally inherited in the Pinaceae. Three cpDNA SSR markers, Pt15169, Pt30204, and Pt71936 (Vendramin and others 1996), are another useful tool to characterize patterns of geographic variation in whitebark pine from the perspective of pollen movement.

The research presented here investigates genetic diversity using isozyme and molecular data, and relates those findings to patterns of genetic variation in key adaptive traits in an earlier seed source study (Mahalovich submitted, Mahalovich and others 2006). Since the majority of the adaptive trait variation occurs within seed sources and the provisional seed zones are conservative in scale, rather than evaluating genetic diversity among and within populations, this study focuses on the amount of genetic diversity among and within seed zones. Project objectives include:

- Determining the genetic structure, genetic diversity, and level of inbreeding among a number of cone collections and branch samples collected from across the Northern Rockies, California, and Nevada;
- Determining whether there is correspondence among provisional seed zone boundaries (Figure 1) using isozyme and molecular data; and
- Developing a comprehensive genetics profile to identify seed sources that are most at risk for being lost due to their apparent uniqueness.

Material and Methods

Material Collection

Low seed yields and a limited geographic representation of bulk seed in inventory narrowed seed lot selection to individual-tree cone collections to achieve a broad geographic sample from eastern California, Idaho, Montana, Nevada, eastern Washington, and Wyoming. A total of 163 individual-tree collections and one bulk lot representing 117 seed source groupings (as determined using common area name and geographic coordinates) are included in this study (Table 1). The bulk lot represents the easternmost population in this collection and is of special interest because of its widespread use for restoration from 2002 to the present, with the last planting of seed from this source anticipated in 2011 (McLaughlin, personal communication). There is no indication from the records that cones were collected from trees of the krummholz growth form.

All seed lots used in Mahalovich and others (2006) were desirable to develop a comprehensive genetics profile of adaptive trait, isozyme and molecular data for each seed source; 79 of the original 110 lots had sufficient seed for this study. When seed quantities were too low or geographic areas were not well represented from the 1991-1997 collections (for example, central ID, NV, northwestern WY), additional seed lots from the

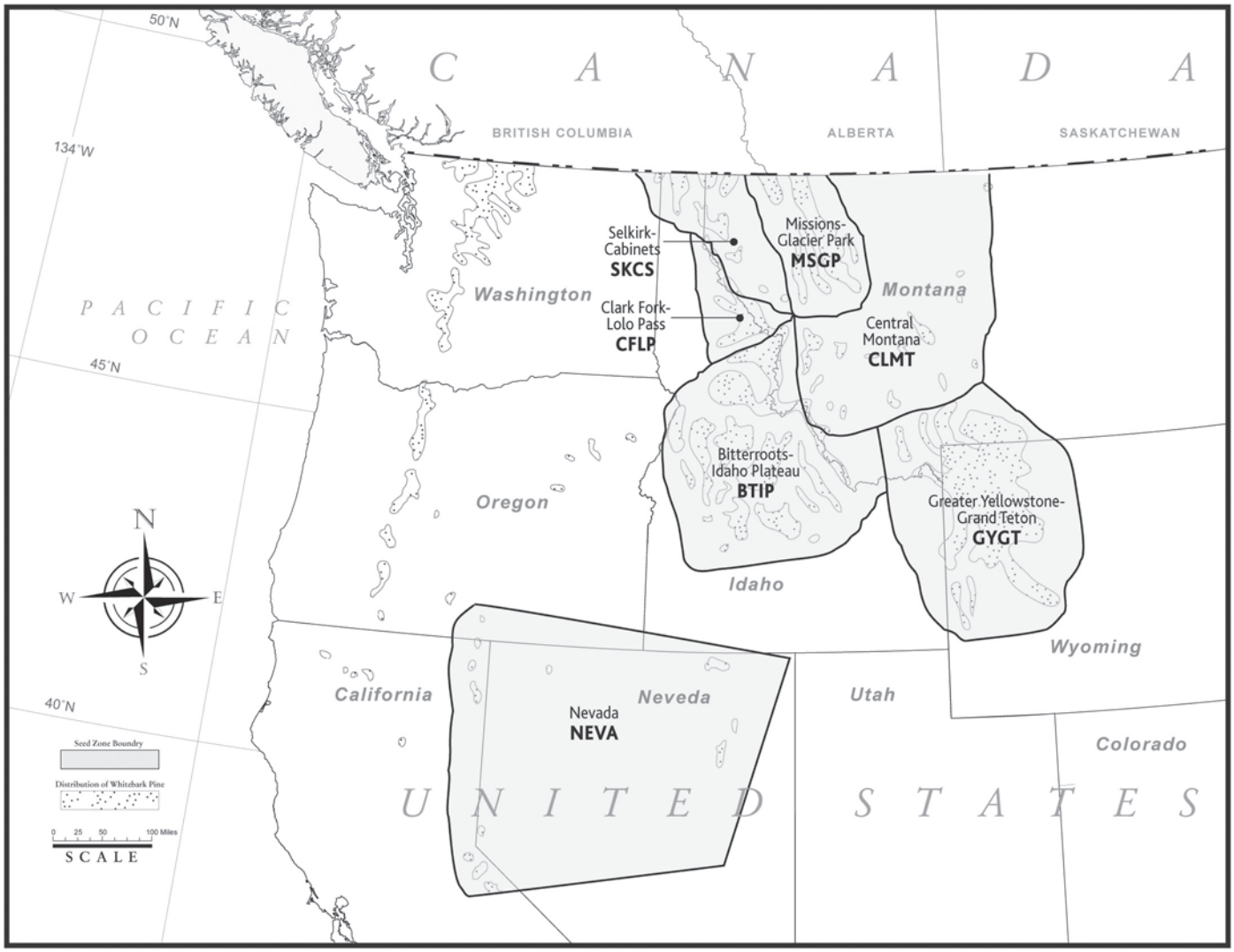

Figure 1. Provisional whitebark pine seed zones in the Inland West overlaying current species distribution (Little 1971).

2001-2006 collections (6000-series plus trees) and 16 vegetative samples from previous research in the Northern Rockies (Richardson 2001) were included. One branch tip and a cone containing five seeds from the oldest known living whitebark pine specimen (1,285 years) located at Railroad Ridge on the Sawtooth National Forest (Perkins and Swetnam 1996) were also included. The authors acknowledge that a representative sample of bulked collections may have better served the parameter estimates for isozyme and molecular data (this paper); however, resources were not available to collect additional bulked collections.

Seed Preparation and Germination

Seed samples were stratified and germinated following Mahalovich and others (2006) and Burr and others (2001). For each germinated seed (10 seeds per collection): (1) the embryo was dissected from the megagametophyte tissue, placed in a microfuge tube, and frozen at -80°C for future analyses, (2) a portion of each of the 10 megagametophytes per collection were placed in a collection tube to achieve a single DNA extraction per collection, effectively genotyping the mother tree through DNA analysis (White and others 2007), and (3) the remaining megagametophyte tissue was placed in an individual well into a microtiter plate for isozyme analysis.

Mitochondrial DNA (mtDNA) Analysis

Amplification of the *nad5a* intron in these samples was completed using primers designed by Wu and others (1998). For each sample, 2.0 ng of mtDNA was amplified following the reaction conditions described by Richardson and others (2002). Amplification was carried out on a MJ Research® PTC-200 thermalcycler (MJ Research, Watertown, Massachusetts, USA). Following amplification the product was purified using the Qiagen™ Qiaquick PCR Purification Kit following the recommended protocols (Qiagen Corp., Valencia, California, USA). Samples were then restricted with *Mse*I. Restriction products were separated via electrophoresis on a 1% agarose gel using 1X TBE buffer (0.045 M Tris-borate, 0.001 M EDTA pH 8) and visualized using ethidium bromide under UV light.

Table 1. Comprehensive genetics profile for the Inland West Whitebark Genetic Restoration Program (percent of blister rust resistance individuals[1], rust resistance family index, late winter cold hardiness and 6-year height ranks described in Mahalovich and others (2006)); by latitude.

National Forest or National Park (NP)	Latitude °N	Longitude °W	Elevation (ft)	Seed Zone[2]	Area	Seed Source Group Number[3]	Proportion Observed Polymorphic Loci[4]	Number of Unique Alleles[4]	No-Spot (%)[5]	Needle Shed (%)	Short Shoot (%)	Bark Reaction (%)	Susceptible (%)	Resistant (%)	Rust Resistance Famil Index Rank	7th-yr Late Winter Cold Hardiness Rank	6-yr Height Rank
Humboldt-Toiyabe	38.08	-119.24	9,146	NEVA	Virginia Lakes	71	0.25	20									
Humboldt-Toiyabe	38.08	-119.24	9,677	NEVA	Dunderberg	72	0.188	19									
Humboldt-Toiyabe	38.08	-119.24	9,745	NEVA	Dunderberg	72	0.313	20									
Humboldt-Toiyabe	38.08	-119.24	9,848	NEVA	Dunderberg	72	0.25	20									
Humboldt-Toiyabe	38.08	-119.24	9,883	NEVA	Virginia Lakes	73	0.25	20									
Humboldt-Toiyabe	38.08	-119.24	9,894	NEVA	Virginia Lakes	73	0.25	20									
Humboldt-Toiyabe	38.08	-119.24	10,117	NEVA	Dunderberg Top	74	0.25	20									
Humboldt-Toiyabe	38.08	-119.24	10,184	NEVA	Dunderberg Top	74	0.188	19									
Humboldt-Toiyabe	38.34	-119.58	9,112	NEVA	Sonora Pass	75	0.313	21									
Humboldt-Toiyabe	38.34	-119.58	9,300	NEVA	Sonora Pass	75	0.375	22									
Humboldt-Toiyabe	38.34	-119.6	9,464	NEVA	Sonora Pass	76	0.125	18									
Humboldt-Toiyabe	38.34	-119.6	9,509	NEVA	Sonora Pass	76	0.375	22									
Humboldt-Toiyabe	38.34	-119.62	9,327	NEVA	Sonora Pass	76	0.125	18									
Humboldt-Toiyabe	39.31	-119.89	8,930	NEVA	Mt. Rose-Tahoe Meadows	77	0.188	19									
Humboldt-Toiyabe	39.31	-119.91	8,677	NEVA	Mt. Rose-Tahoe Meadows	77	0.438	23									
Humboldt-Toiyabe	39.31	-119.91	8,968	NEVA	Mt. Rose Trailhead	78	0.188	19									
Humboldt-Toiyabe	39.31	-119.91	8,968	NEVA	Mt. Rose Trailhead	78	0	16									
Humboldt-Toiyabe	39.32	-119.91	8,587	NEVA	Mt. Rose-Tamarack Lake	79	0.25	20									
Humboldt-Toiyabe	39.32	-119.91	8,640	NEVA	Mt. Rose-Tamarack Lake	79	0.375	22									
Humboldt-Toiyabe	39.44	-119.96	8,560	NEVA	Garson Road	80	0.375	22									
Humboldt-Toiyabe	39.44	-119.96	8,596	NEVA	Garson Road	80	0.25	20									
Shoshone	42.64	-109.7	10,200	GYGT	Blue Ridge	81	0.375	22									
Bridger-Teton	43.48	-109.94	8,680	GYGT	Jackson Hole Mountain Resort	82	0.125	18									
Shoshone	43.5	-109.86	9,590	GYGT	Union Pass	83	0.188	19									
Shoshone	43.51	-109.84	9,800	GYGT	Wind River Ranger District	70	0.375	22	0.112	0.045	0.119	0.097	0.627	0.373	57.5	16	58
Bridger-Teton	43.61	-110.26	9,400	GYGT	Gunsight Pass	84	n/a	n/a									
Bridger-Teton	43.61	-110.26	9,400	GYGT	Gunsight Pass #1	84	n/a	n/a									
Bridger-Teton	43.61	-110.86	9,085	GYGT	Labarge Creek	85	0.25	20									
Boise	43.61	-115.45	8,300	BTIP	Trinity	86	0.188	19									
Grand Teton NP	43.68	-110.8	9,080	GYGT	Stewarts Draw	87	0.313	21									
Shoshone	43.73	-109.6	9,512	GYGT	Bog Lakes	88	0.313	21									
Caribou-Targhee	43.78	-110.95	9,000	GYGT	Grand Targhee Ski Resort	89	n/a	n/a									
Caribou-Targhee	43.79	-110.93	8,000	GYGT	East Dry Creek	90	0.063	17									
Caribou-Targhee	43.79	-110.93	9,530	GYGT	Grand Targhee	91	0.125	18									
Bridger-Teton	43.82	-110.21	8,520	GYGT	Togwater Lodge #1	92	n/a	n/a									
Sawtooth	43.88	-114.72	8,880	BTIP	Galena Summit	93	0.313	21									
Sawtooth	44.05	-114.66	9,520	BTIP	4th of July	94	0.125	18									
Sawtooth	44.1	-114.6	9,440	BTIP	Railroad Ridge	95	0	13									
Boise	44.19	-115.76	7,830	BTIP	Scott Mountain	96	0.188	19									
Caribou-Targhee	44.53	-111.69	8,100	GYGT	Boatman Springs	97	0.25	20									
Caribou-Targhee	44.54	-111.61	8,100	BTIP	Keg Springs	98	0.188	19									
Caribou-Targhee	44.54	-111.43	8,200	GYGT	Mt. Sawtelle #1	99	n/a	n/a									
Caribou-Targhee	44.54	-111.43	8,200	GYGT	Mt. Sawtelle #2	99	n/a	n/a									
Caribou-Targhee	44.54	-111.41	8,350	GYGT	Sawtel Peak	1	0.25	20	0.129	0.007	0.107	0.021	0.736	0.264	95.5	20	78
Caribou-Targhee	44.55	-111.43	8,600	BTIP	Sawtell Mountain	1	0.25	20									
Yellowstone NP	44.81	-110.44	8,936	GYGT	Washburn	100	0.375	22									
Beaverhead-Deerlodge	44.82	-111.87	8,800	GYGT	W.F. Cabin	2	0.188	19	0.167	0.08	0.167	0.029	0.558	0.442	74		56
Beaverhead-Deerlodge	44.82	-111.87	8,800	GYGT	W.F. Cabin	2	0.313	21	0.186	0.062	0.142	0.062	0.549	0.451	46		76
Shoshone	44.95	-109.47	10,000	GYGT	Beartooth Pass #1	101	n/a	n/a									
Shoshone	44.95	-109.47	10,000	GYGT	Beartooth Pass #2	101	n/a	n/a									
Payette	45	-116.14	7,200	BTIP	Brundage Mountain	102	0.188	19									
Gallatin	45.03	-109.95	9,018	CLMT	Miller Trail	103	0.313	21									
Custer	45.04	-109.43	8,900	GYGT	Hellroaring I	4	0.375	23	0.087	0.022	0.043	0.065	0.783	0.217	81	33	104
Custer	45.04	-109.45	9,200	GYGT	Hellroaring II	5	0	14	0.07	0.023	0.047	0.093	0.767	0.233	87		91
Custer	45.04	-109.56	8,900	GYGT	Hellroaring I	4	0.313	21	0.064	0	0.064	0.043	0.83	0.17	110	43	68
Gallatin	45.05	-109.95	9,600	GYGT	Daisy Pass	6	0.313	21	0.087	0	0	0.043	0.87	0.13	95.5		100
Beaverhead-Deerlodge	45.15	-113.55	8,400	CLMT	Goldstone Pass	7	0.25	20	0.068	0	0.136	0	0.795	0.205	106	28	97
Beaverhead-Deerlodge	45.15	-113.55	8,400	CLMT	Goldstone Pass	7	0.313	21	0.113	0.05	0.149	0.035	0.652	0.348	44.5		19
Beaverhead-Deerlodge	45.15	-113.55	8,400	CLMT	Goldstone Pass	7	0.313	21	0.021	0.021	0.104	0.021	0.833	0.167	85		83

Table 1. *Continued.*

National Forest or National Park (NP)	Latitude °N	Longitude °W	Elevation (ft)	Seed Zone[2]	Area	Seed Source Group Number[3]	Proportion Observed Polymorphic Loci[4]	Number of Unique Alleles[4]	No-Spot (%)[5]	Needle Shed (%)	Short Shoot (%)	Bark Reaction (%)	Susceptible (%)	Resistant (%)	Rust Resistance Famil Index Rank	7th-yr Late Winter Cold Hardiness Rank	6-yr Height Rank
Beaverhead-Deerlodge	45.15	-113.55	8,400	CLMT	Goldstone Pass	7	0.25	20	0.149	0	0.064	0.064	0.723	0.277	29		30
Beaverhead-Deerlodge	45.15	-113.55	8,400	CLMT	Goldstone Pass	7	0.375	22	0.183	0.12	0.204	0.042	0.451	0.549	17		49
Payette	45.22	-116.12	7,800	BTIP	Lava Ridge	104	0.438	23									
Gallatin	45.27	-111.42	9,000	GYGT	Lone Mountain	8	0.25	20	0.125	0.014	0.063	0.083	0.715	0.285	86	11	64
Nez Perce	45.36	-116.51	8,000	BTIP	Riggins	10	0.438	23	0.182	0.015	0.106	0.061	0.636	0.364	25		72
Nez Perce	45.38	-116.48	8,000	BTIP	Heavens Gate	11	0.313	21	0.182	0.045	0.227	0.045	0.5	0.5	41		101
Gallatin	45.4	-111.28	8,600	GYGT	Little Bear	14	0.313	21	0.225	0.099	0.12	0.035	0.521	0.479	67	13	60
Salmon-Challis	45.47	-114.29	7,860	BTIP	Tin Cup	16	0.188	19	0.191	0.085	0.227	0.085	0.411	0.589	20	35	16
Salmon-Challis	45.47	-114.29	7,860	BTIP	Tin Cup	16	0.188	19	0.237	0.017	0.186	0.051	0.508	0.492	77.5		103
Nez Perce	45.63	-115.95	8,200	BTIP	Gospel Peak	17	0.125	18	0.064	0.05	0.329	0.071	0.486	0.514	36.5		33.5
Beaverhead-Deerlodge	45.71	-112.93	8,000	CLMT	Quartz Hill	18	0.375	22	0.05	0.014	0.05	0.029	0.856	0.144	104	4	85
Nez Perce	45.71	-115	8,200	BTIP	Burnt Knob	19	0.188	19	0.133	0.119	0.315	0.056	0.378	0.622	42	41	25
Bitterroot	45.72	-113.99	8,270	CLMT	Saddle Mountain	20	0.188	19	0.263	0.113	0.165	0.023	0.436	0.564	59		38
Bitterroot	45.72	-113.99	8,270	CLMT	Saddle Mountain	20	0.375	22	0.133	0.035	0.077	0.021	0.734	0.266	91	19	24
Gallatin	45.89	-110.96	8,861	CLMT	Corbly Gulch	105	0.5	24									
Nez Perce	45.91	-115.71	7,140	BTIP	Pilot Knob	22	0.438	23	0.22	0.073	0.165	0.147	0.394	0.606	10	40	42
Nez Perce	45.91	-115.71	7,140	BTIP	Pilot Knob	22	0.125	18	0.141	0.049	0.366	0.141	0.303	0.697	2	5	80
Nez Perce	45.91	-115.07	6,940	BTIP	Elk Mountain	106	0.125	18									
Beaverhead-Deerlodge	45.94	-113.51	7,900	CLMT	Mudd Ridge	23	0.25	20	0.104	0.042	0.042	0.042	0.771	0.229	72		2
Bitterroot	46.07	-113.8	8,040	CLMT	Coyote Meadows	24	0.375	22	0.161	0.073	0.095	0.036	0.635	0.365	73	17	61
Bitterroot	46.07	-113.8	8,040	CLMT	Coyote Meadows	24	0.188	19	0.118	0.022	0.074	0.022	0.765	0.235	83	26	75
Bitterroot	46.07	-113.8	8,040	CLMT	Coyote Meadows	24	0.438	23	0.117	0.146	0.08	0.051	0.606	0.394	57.5	17	35
Clearwater	46.3	-114.61	7,400	BTIP	Diablo Mountain	26	0.438	23	0.156	0.067	0.356	0.067	0.356	0.644	12		32
Beaverhead-Deerlodge	46.39	-112.19	7,600	CLMT	Occidental Plateau	28	0.125	18	0.294	0.118	0.059	0.059	0.471	0.529	15		63
Helena	46.4	-111.17	7,500	CLMT	Mt. Edith	107	0.313	21									
Clearwater	46.45	-114.73	6,895	BTIP	Tom Beal Park	108	0.188	19									
Bitterroot	46.51	-114.22	6,470	CLMT	St. Mary's Peak	30	0.125	18	0.134	0.045	0.08	0.018	0.723	0.277	101	18	59
Bitterroot	46.51	-114.22	6,470	CLMT	St. Mary's Peak	30	0.375	22	0.117	0.058	0.109	0.058	0.657	0.343	66		18
Clearwater	46.53	-115	7,000	CFLP	Indian Post Off.	31	0.25	20	0.2	0.096	0.207	0.104	0.393	0.607	34		77
Clearwater	46.55	-114.5	6,970	BTIP	Beaver Meadows	109	0.313	21									
Clearwater	46.56	-114.44	7,300	CFLP	Beaver Ridge	32	0.25	20	0.134	0.071	0.17	0.116	0.509	0.491	44.5		40
Clearwater	46.56	-114.44	7,300	CFLP	Beaver Ridge	32	0.438	23	0.125	0.059	0.272	0.007	0.537	0.463	103	38	28
Clearwater	46.56	-114.44	7,300	CFLP	Beaver Ridge	32	0.313	20	0.174	0.094	0.377	0.022	0.333	0.667	30.5	10	13
Clearwater	46.56	-114.44	7,300	CFLP	Beaver Ridge	32	0.25	20	0.022	0.058	0.18	0.101	0.64	0.36	79		73
Clearwater	46.61	-114.78	6,970	CFLP	Lost Lake Trail #3	110	0.188	19									
Clearwater	46.64	-114.86	7,200	CFLP	Blacklead Mountain	34	0.438	23	0.336	0.044	0.239	0.097	0.283	0.717	3	3	81
Clearwater	46.64	-114.86	7,200	CFLP	Blacklead Mountain	34	0	15	0.261	0.085	0.183	0.127	0.345	0.655	4	15	51
Lewis & Clark	46.77	-110.6	8,068	CLMT	Ant Park	111	0.375	22									
Lewis & Clark	46.77	-110.62	8,073	CLMT	Higgins Park	112	0.375	22									
Clearwater	46.87	-115.25	6,200	CFLP	Indian Henry Ridge	113	0.125	18									
Bridger-Teton	46.97	-107.09	9,028	GYGT	Fish Creek	114	0.375	22									
Clearwater	46.99	-115.06	6,556	CFLP	Long Creek Ridge	115	0.188	19									
Idaho Panhandle	47.	-116.03	5,800	CFLP	Freezeout	36	0.25	20	0.429	0	0.429	0	0.143	0.857	61.5		79
Idaho Panhandle	47.	-116.03	5,940	CFLP	Freezeout	36	0.313	21	0.415	0.128	0.16	0.043	0.255	0.745	13	21	107
Idaho Panhandle	47.01	-116.07	5,600	CFLP	Marks Butte	116	n/a	n/a									
Lolo	47.01	-114.01	7,860	MSGP	Snow Bowl	37	0.125	18	0.087	0.007	0.181	0.08	0.645	0.355	76	39	43
Idaho Panhandle	47.01	-116.03	5,920	CFLP	Freezeout	38	0.25	20	0.208	0.042	0.375	0.083	0.292	0.708	26		11
Idaho Panhandle	47.01	-116.03	5,920	CFLP	Freezeout	38	0.375	22	0.081	0.067	0.422	0.089	0.341	0.659	64	52	95
Lolo	47.03	-113.99	7,284	SKCS	Point Six	39	0.25	20	0.321	0.122	0.374	0.046	0.137	0.863	9	25	65
Idaho Panhandle	47.09	-115.27	6,707	CFLP	Simmons Peak Look Out	117	0.188	19									
Lolo	47.09	-114.57	7,420	SKCS	Stark Mountain	42	0.188	19	0.278	0.083	0.226	0.098	0.316	0.684	16		105
Helena	47.11	-112.73	8,400	CLMT	Red Mountain	118	0.188	19									
Lolo	47.15	-115.25	6,972	SKCS	Little Joe Mountain	43	0.375	22	0.076	0.069	0.313	0.097	0.444	0.556	33		9
Lolo	47.15	-115.25	6,925	BTIP	Stark Mountain	119	0.25	20									
Lolo	47.16	-114.37	6,960	SKCS	Squaw Peak	44	0.25	20	0.093	0.047	0.163	0.116	0.581	0.419	47		87
Lolo	47.16	-115.25	7,050	SKCS	Little Joe Mountain	43	0.25	20	0.095	0.048	0.286	0.095	0.476	0.524	11		1
Idaho Panhandle	47.19	-116.05	5,880	CFLP	Freezeout	46	0.125	18	0.196	0.174	0.116	0.058	0.457	0.543	19		54
Lolo	47.19	-113.34	7,275	MSGP	Morrell Look Out	120	0.188	19									
Lolo	47.19	-113.34	7,300	MSGP	Morrell Look Out	120	0.25	20									

Table 1. *Continued.*

National Forest or National Park (NP)	Latitude °N	Longitude °W	Elevation (ft)	Seed Zone[2]	Area	Seed Source Group Number[3]	Proportion Observed Polymorphic Loci[4]	Number of Unique Alleles[4]	No-Spot (%)[5]	Needle Shed (%)	Short Shoot (%)	Bark Reaction (%)	Susceptible (%)	Resistant (%)	Rust Resistance Famil Index Rank	7th-yr Late Winter Cold Hardiness Rank	6-yr Height Rank
Lolo	47.19	-113.37	7,670	MSGP	Morrell Look Out	121	0.125	18									
Lolo	47.19	-113.37	7,796	MSGP	Morrell Lookout	121	n/a	n/a									
Idaho Panhandle	47.27	-115.49	6,150	CFLP	Craddock	122	0.25	20									
Idaho Panhandle	47.29	-115.53	6,414	CFLP	Quarrles Peak	123	0.125	18									
Lewis & Clark	47.37	-112.75	6,724	MSGP	Crown Mtn	124	0.375	22									
Lewis & Clark	47.39	-112.75	6,859	MSGP	Crown Mtn	124	0.25	20									
Lolo	47.52	-115.71	6,405	SKCS	Burke Summit	47	0.25	20	0.268	0.042	0.394	0.042	0.254	0.746	27	49	29
Lewis & Clark	47.52	-112.8	7,600	MSGP	Sheep Shed Mountain	48	0.313	21	0.077	0.07	0.317	0.021	0.514	0.486	75		14
Lolo	47.52	-115.7	6,150	SKCS	Burke Summit	49	0.25	20	0.229	0.063	0.333	0.042	0.333	0.667	43		5
Kootenai	47.65	-115.74	5,650	CFLP	Beaver Creek	50	0.125	18	0.113	0.057	0.284	0.135	0.411	0.589	8	47	27
Kootenai	47.65	-115.74	5,650	CFLP	Beaver Creek	50	0.188	18	0.238	0.033	0.082	0.082	0.566	0.434	32		48
Kootenai	47.65	-115.74	5,650	CFLP	Beaver Creek	50	0.125	20	0.188	0.104	0.229	0.083	0.396	0.604	6.5	42	57
Lolo	47.75	-114.85	6,140	SKCS	Thompson Peak	51	0.063	17	0.161	0.07	0.308	0.021	0.441	0.559	63	36	10
Lolo	47.77	-115.32	6,200	SKCS	Vermillion Pass #1	125	n/a	n/a									
Flathead	47.78	-113.72	6,550	MSGP	Napa Point #2	126	n/a	n/a									
Kootenai	47.83	-115.39	5,928	SKCS	7-Point Mountain	52	0.25	20	0.167	0.014	0.181	0.069	0.569	0.431	35		8
Lewis & Clark	47.84	-112.81	7,500	MSGP	Our Lake	53	0.313	21	0	0	0.4	0	0.6	0.4	94		98
Kootenai	47.95	-115.56	6,000	SKCS	Baree Mountain	55	0.313	21	0.194	0.032	0.226	0.032	0.516	0.484	68.5	30	7
Flathead	48.19	-113.35	6,480	MSGP	25-Mile	56	0.25	20									
Flathead	48.19	-113.35	6,600	MSGP	25-Mile	56	0.25	20									
Idaho Panhandle	48.35	-116.75	5,430	SKCS	Gisborne	57	n/a	n/a	0.167	0	0.583	0.083	0.167	0.833	14		23
Idaho Panhandle	48.37	-116.2	6,370	SKCS	Lunch Peak	58	0.188	19	0.228	0.162	0.309	0.066	0.235	0.765	24		71
Idaho Panhandle	48.38	-116.19	6,200	SKCS	Lunch Peak	58	n/a	n/a									
Flathead	48.44	-113.97	6,200	MSGP	Desert Mountain	127	0.313	21									
Flathead	48.49	-114.34	6,000	MSGP	Big Mountain	60	0.313	21	0.185	0.054	0.3	0.077	0.385	0.615	22		31
Flathead	48.49	-114.34	6,500	MSGP	Big Mountain	61	0.188	19	0.135	0.027	0.162	0.027	0.649	0.351	70.5	29	67
Flathead	48.49	-114.34	6,000	MSGP	Big Mountain	60	0.438	23	0.132	0.081	0.184	0.081	0.522	0.478			41
Flathead	48.49	-114.34	6,000	MSGP	Big Mountain #2	60	n/a	n/a									
Flathead	48.49	-114.34	6,810	MSGP	Big Mountain #1	61	n/a	n/a									
Flathead	48.54	-114.14	5,400	MSGP	Nicola	128	0.188	19									
Flathead	48.55	-114.23	6,200	MSGP	Standard	129	0.125	18									
Kootenai	48.57	-115.72	5,700	SKCS	Huson Peak	130	0.125	18									
Colville	48.71	-118.47	7,135	SKCS	Copper Butte	61	0.313	21	0.087	0.065	0.217	0.022	0.609	0.391	97	50	106
Colville	48.71	-118.47	7,137	SKCS	Copper Butte	61	0.188	19	0.158	0	0.158	0	0.684	0.316	105		108
Idaho Panhandle	48.83	-116.6	6,800	SKCS	Pyramid Pass	62	0.375	22	0.304	0.13	0	0.043	0.522	0.478	28		17
Idaho Panhandle	48.84	-116.51	6,700	SKCS	Farnham Ridge	63	0.125	18	0.092	0.042	0.155	0.028	0.683	0.317	82	42	33.5
Idaho Panhandle	48.86	-116.47	5,820	SKCS	Farnham Peak	64	0.25	20	0.12	0.174	0.283	0.12	0.304	0.696	38		82
Colville	48.88	-117.24	6,480	SKCS	Sullivan Look Out	65	0.25	20	0.182	0.091	0.205	0.091	0.432	0.568	6.5	46	66
Glacier NP	48.88	-114.49	6,000	MSGP	Siyeh Bend	131	0.25	20									
Flathead	48.88	-114.49	6,450	MSGP	Big Mountain	66	0.375	22	0.056	0.028	0.141	0.056	0.718	0.282	88		15
Flathead	48.88	-114.51	6,000	MSGP	Hornet Mountain	67	0.563	25	0.022	0.043	0.087	0.022	0.826	0.174	70.5	22	46
Kootenai	48.93	-114.79	5,800	MSGP	Foundation Creek	132	0.313	21									
Colville	48.97	-117.11	6,800	SKCS	Salmo Lookout	68	0.375	22	0.34	0.064	0.128	0.255	0.213	0.787	1	7	102
Kootenai	48.97	-115.84	7,200	SKCS	NW Peaks	69	0.438	23	0.174	0.13	0.13	0	0.565	0.435	52	48	21
Kootenai	48.98	-114.7	5,400	MSGP	Frozen Lake	133	0.25	20									

[1] No-spot (%) = no spot symptoms on needles, no canker development, only trait to infer immunity; Needle shed (%) = seedlings drop their infected (spotted) needles less than 12 months after inoculation and before the mycelium reaches the stem; Short shoot (%) = seedlings retain their infected (spotted) needles beyond 12 months after inoculation, but never develop a canker; mycelium do not enter woodier bark tissue at the base of the needle fascicle or junction of the short shoot and needle fascicle bundle; Bark reactions (%) = increased number of callus formation on branches and stems, walling off cankers, and thereby preventing further infection.

[2] Seed zone designations: BTIP=Bitterroots-Idaho Plateau, CFLP=Clark Fork-Lolo Pass, CLMT=Central Montana, GYGT=Greater Yellowstone-Grand Teton, MSGP=Missions-Glacier Park, NEVA=Nevada, SKCS=Selkirk-Cabinets.

[3] Seed source group number determined using common area name and geographic coordinates.

[4] Isozyme data not available from branch samples.

[5] Blister rust resistence, late winter cold hardiness or height data added at the conmpletion of each rust screening.

Isozymes

Megagametophyte tissue was homogenized in phosphate buffer and absorbed onto 2 mm wide paper wicks. Starch gel (11% w/v) electrophoresis revealed 16 loci in three buffer systems that resolved strong enzyme activity in all tissue analyzed (USDA Forest Service 2003). Four loci were resolved in a lithium borate electrode buffer-tris citrate gel buffer combination (system LB): leucine aminopeptidase (LAP1 and LAP2; EC 3.4.11.1), phosphoglucomutase (PGM; EC 5.4.22), and phosphoglucose isomerase (PGI2; EC 5.3.1.9). Five loci were resolved in a sodium borate electrode buffer-tris citrate gel buffer combination (system SB): aspartate aminotransferase (AAT1, AAT2 and AAT3; EC 2.6.1.1), uridine diphosphoglucose pyrophosphorylase (UGPP; EC 2.7.7.9), and triose-phosphate isomerase (TPI; EC 5.3.1.1). Seven loci were resolved in a morpholine citrate electrode and gel buffer, pH 8 (system MC8): phosphogluconate dehydrogenase (6PGD; EC 1.1.1.44), isocitrate dehydrogenase (IDH; EC 1.1.1.42), shikimic acid dehydrogenase (SKD1 and SKD2; EC 1.1.1.25), and malate dehydrogenase (MDH1, MDH2 and MDH3; EC 1.1.1.37). Following incubation in ten substrate specific stains, genotypic data were collected for 16 loci (PGM, LAP-1 and 2, PGI-2, AAT-1, 2, and 3, UGPP, TPI, SKD-1 and 2, IDH, 6PGD, MDH-1, 2, and 3). Because of the condition of the 16 samples of branch tissue upon arrival at the lab, no isozyme activity was present in these tissues.

The presence of seed from at least two trees in one sample was detected in four collections (three samples from 'Coyote Meadows' and one from 'Blue Ridge'). These seed lots could be the result of seed contamination from the point of field collections to seed processing. When more than two alleles were detected for a sample at a locus, the least common allele at each three-allele score was discarded so that the samples could be used in the analysis. Of the 147 multilocus isozyme genotypes generated, no two samples matched (resulting in 147 unique genotypes).

DNA Extraction and Amplification

DNA was extracted from each sample using the Qiagen DNEasy 96-well format protocol. Tissue was first homogenized in a collection tube, AP1 buffer added, the sample re-homogenized on the MixerMill, then an aliquot of slurry transferred to a new collection tube for extraction. The remaining slurry was frozen for additional extractions if needed. DNA concentrations were quantified using fluorometry with pico-green.

Though isozyme activity was not present in the 16 samples of branch tissue, it was still possible to extract DNA from each sample using the Qiagen DNEasy-Mini protocol. Approximately 150 mg of needle tissue per sample was ground by hand under liquid nitrogen in a mortar and pestle and DNA extracted following Qiagen DNEasy kit instructions. DNA concentrations were quantified using fluorometry with pico-green. DNA quality was further checked by electrophoresing each sample on a 0.8% agarose gel (1X TBE), staining with ethidium bromide and visualizing under UV light.

Chloroplast DNA (cpDNA) Analysis

Polymerase chain reaction (PCR) amplification was performed simultaneously for three loci: Pt15169, Pt30204, and Pt71936 (Vendramin and others 1996) in 25-µl reaction volumes containing 10 ng cpDNA, 0.2 µM of each primer, and 12.5 ul of the Qiagen multiplex PCR mix including *Taq* DNA polymerase. The PCRs were completed using the following protocol on PTC-200 thermal cyclers: 15 minutes at 95 °C; 30 cycles of 30 seconds at 94 °C, one minute 30 seconds at 57 °C, and one minute at 72 °C; 30 minutes at 60 °C; followed by an indefinite hold at 4 °C. The resulting PCR products were separated on an ABI Prism 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA), as recommended by the manufacturer. Peaks were sized and binned, with alleles called using GeneMarker 1.51 (SoftGenetics, State College, PA, USA), with GS (500-250) ROX as an internal size standard for each sample.

Three cpDNA SSR markers characterize 164 samples. The three Coyote Meadows samples were removed from the analysis because they show the presence of seed from at least two trees in each collection. The bulk collection from Wind River was removed because this sample could not be analyzed within this dataset. The Blue Ridge sample also shows evidence of containing seed from at least two trees; but instead of discarding this sample, it was possible to artificially double the information because only one SSR locus shows the presence of the alternate tree.

Data Analysis

Analysis of mtDNA data was completed by assigning a haplotype to each individual, based on the presence of a single band (haplotype 1) or two bands (haplotype 2). Descriptive statistics for isozyme and cpDNA data were estimated with the GDA program (Lewis and Zaykin 2001), GenAlEx-6 (Peakall and Smouse 2005), and Popgene (Yeh and others 1997). The following parameters were estimated to describe the observed genetic diversity: percent polymorphic loci (P), number of alleles per locus (Na), number of alleles per locus with a frequency greater than 5 percent (Na Freq > 5 percent), effective number of alleles (N_e), information index (equivalent to the Shannon-Weaver Index, I), number of alleles unique to a seed zone (number of private alleles), unbiased expected heterozygosity (H_e), observed heterozygosity (H_o), number of migrants (N_m), fixation index (F), and outcrossing rate (t).

Pairwise measures of the fixation index (F_{ST}) quantify the distribution of genetic diversity among seed zones. Nei's genetic distance (1972) was estimated for all pairs of zones using the program GenAlEx-6 (Peakall and Smouse 2005). GenAlEx-6's test of isolation by distance was employed using the pairwise F_{ST} values (based on isozyme data), with significance determined using the Mantel test. Genetic differentiation among seed sources for survival, rust resistance,

late winter cold hardiness and early height-growth was estimated for all traits (Mahalovich submitted) by calculating the index $Q_{ST} = \sigma^2_p/(\sigma^2_p + 2 \sigma^2_a)$, where σ^2_p is the among seed source variance and σ^2_a is the within-population additive genetic variance (Spitze 1993). The F_{ST} values among seven seed zones were then compared to the Q_{ST} indices to examine which evolutionary forces may be operating in the Inland West.

Principal component analysis, cluster analysis and discriminant analysis of the continuous variables (chloroplast (cpDNA) SSR markers and geographic variables) were performed using the statistical program R (R Foundation http://www.r-project.org).

Population phenograms (Figure 3) were constructed to describe the ancestral relationship among samples, using predefined seed zone assignments (Figure 1). Phenograms were built separately from Nei's genetic distance (1972) estimated from the isozyme and cpDNA data sets, using Neighbor-Joining methods; significance of branches was determined over 1,000 bootstrap replicates using the majority rule extended method to build the consensus tree, as employed by the package PHYLIP (Felsenstein 2005). Principal Coordinate Analysis (GenAlEx-6; Peakall and Smouse 2005) was used to find and plot the major patterns among predefined zones.

Results

Mitochondrial DNA (mtDNA) Variation

Variation identified in the *nad5a* intron of the mitochondrial genome in whitebark pine results in two haplotypes or genetic variants (Richardson and others 2002). Of the 117 collection sites represented, 108 of them contain haplotype 1, while the remaining nine contain haplotype 2. Haplotype 1 is present in the Idaho, Montana, eastern Washington and Wyoming collections. Haplotype 2 is characteristic of the eastern California and Nevada collections. Based on these two haplotypes, the Nevada zone is genetically differentiated (separated) from the remaining zones in the Northern Rockies.

Isozyme Variation

Genetic Diversity: Isozyme data from 16 loci were generated from 147 samples. Levels of genetic diversity are high within these whitebark samples: percent polymorphic loci or P = 100%; number of alleles or Na = 4.0; and expected heterozygosity or H_e = 0.271 (Table 2). Similar levels of diversity occur within each zone, where zone means are also high: P = 88.4%; Na = 2.5; H_e = 0.263. Mean fixation index (F) is 0.01, and for all trees within a zone range from -0.059 to 0.076, indicating general random mating with no marked excess of heterozygosity or inbreeding. Mean outcrossing rate (t) is 0.98, and for trees within a zone range from 0.858 to 1.125, indicating a lack of inbreeding.

Genetic Structure: Of the total variation measured, very little is among zones, indicating zones share a large degree of genetic similarity. Analysis of Molecular Variance (AMOVA) indicates that 99 percent of the variation measured is within zones (only 1 percent is among zones). Wright's (1951) inbreeding coefficient within individuals relative to zones (F_{IS}) = -0.016 and the inbreeding coefficient within individuals relative to the total (F_{IT}) = 0.011. F_{ST} (genetic differentiation among populations, or in this study, the proportion of the total diversity that separates the zones) is only 2.6 percent. Low F_{IS} (-0.016) and F_{IT} (0.011) values and a high number of migrants per generation (N_m = 9.354) also indicate a lack of inbreeding. Nei's (1972) genetic identity (I) in this study is a measure of genetic similarity between zones, with a value of 100 percent meaning that two zones share the same alleles in the same frequencies, and a value of 0 meaning that two zones have no allele in common. Identity values range from 97.7 to 99.4 percent (Table 3 lower triangle) confirming high genetic similarity among zones. Although all zones are very similar to one other, the Clark Fork-Lolo Pass and Missions-Glacier Park

Table 2. Genetic diversity statistics for Inland West whitebark pine samples based on 16 isozyme loci. N = sample size; P = percent polymorphic loci; Na = number of alleles; N_e = effective number of alleles; I = information index; H_e = expected heterozygosity; H_o = observed heterozygosity; F = fixation index; t = outcrossing rate.

Zone (Standard Errors)	Bitterroots-Idaho Plateau		Clark Fork-Lolo Pass		Central Montana		Greater Yellowstone-Grand Teton		Missions-Glacier Park		Nevada		Selkirk-Cabinets		Zone Mean		All Samples	
N	20.9	(0.781)	20.6	(0.176)	20.5	(0.195)	20.5	(0.176)	20.8	(0.127)	21	(0)	19.8	(0.127)	20.5	(0.398)	147	(0.532)
P	100	——-	93.8	——-	93.8	——-	75	——-	87.5	——-	81.3	——-	87.5	——-	88.4	——-	100	——-
Na	2.7	(0.270)	2.4	(0.180)	2.8	(0.262)	2.5	(0.316)	2.5	(0.258)	2.4	(0.258)	2.4	(0.203)	2.5	(0.160)	4	(0.400)
Na Freq. >= 5%	21.9	(0.180)	1.9	(0.180)	1.9	(0.202)	1.8	(0.209)	1.9	(0.202)	1.9	(0.180)	2.1	(0.193)	1.9	(0.090)	1.9	(0.180)
N_e	1.5	(0.112)	1.4	(0.135)	1.6	(0.136)	1.5	(0.125)	1.5	(0.131)	1.4	(0.121)	1.4	(0.103)	1.5	(0.076)	1.5	(0.119)
I	0.495	(0.076)	0.416	(0.078)	0.508	(0.086)	0.447	(0.092)	0.464	(0.086)	0.441	(0.083)	0.457	(0.073)	0.461	(0.032)	0.496	(0.078)
Number of Private Alleles	0.25	(0.112)	0.063	(0.063)	0.125	(0.085)	0.188	(0.136)	0.063	(0.063)	0.313	(0.151)	0.125	(0.085)	0.152	(0.094)	4	(0.428)
H_e	0.283	(0.048)	0.238	(0.051)	0.287	(0.054)	0.254	(0.055)	0.264	(0.053)	0.25	(0.049)	0.263	(0.046)	0.263	(0.018)	0.271	(0.049)
H_o	0.251	(0.049)	0.234	(0.048)	0.309	(0.074)	0.266	(0.064)	0.28	(0.058)	0.253	(0.050)	0.257	(0.047)	0.264	(0.024)	0.264	(0.052)
F	0.076	(0.062)	-0.025	(0.022)	-0.049	(0.044)	-0.051	(0.014)	-0.059	(0.029)	-0.013	(0.029)	-0.008	(0.044)	-0.018	(0.046)	0.01	(0.019)
t	0.858	(0.091)	1.051	(0.077)	1.103	(0.082)	1.107	(0.064)	1.125	(0.084)	1.026	(0.072)	1.016	(0.063)	1.036	(0.069)	0.98	(0.045)

Table 3. Pairwise zone matrix of Nei's genetic identity (lower triangle) and FST (upper triangle) for Inland West whitebark pine samples based on 16 isozyme loci.

Zone	Bitterroots-Idaho Plateau	Clark Fork-Lolo Pass	Central Montana	Greater Yellowstone-Grand Teton	Missions-Glacier Park	Nevada	Selkirk-Cabinets
Bitterroots-Idaho Plateau		0.017	0.011	0.016	0.013	0.013	0.018
Clark Fork-Lolo Pass	0.986		0.016	0.020	0.010	0.022	0.012
Central Montana	0.992	0.988		0.011	0.012	0.011	0.017
Greater Yellowstone-Grand Teton	0.988	0.986	0.991		0.015	0.019	0.019
Missions-Glacier Park	0.988	0.994	0.990	0.990		*0.025	0.011
Nevada	0.990	0.982	0.991	0.985	0.977		*0.026
Selkirk-Cabinets	0.987	0.992	0.985	0.989	0.992	0.978	

* = significance at the 5% level of probability.

are most similar. The two least similar zones are Selkirk-Cabinets and Nevada, followed closely by Missions-Glacier Park and Nevada. F_{ST} values show similar trends of high genetic similarity and weak differentiation between zones, with estimates ranging from 1.0 to 2.6 percent (Table 3 upper triangle).

Principal Coordinate Analysis of the isozyme data shows that the Missions-Glacier Park, Selkirk-Cabinets, and Clark Fork-Lolo Pass zones cluster somewhat together and the Central Montana and Bitterroots-Idaho Plateau zones cluster (Figure 2a). The Greater Yellowstone-Grand Teton and Nevada zones occur by themselves. The percentage of variation explained by the first two principal coordinates is 62.8 percent (the first three, principal coordinates explain 77.0 percent of the total variation). Mid-point phenograms (Figure 3a) show similar zonal relationships (cluster Bitterroots-Idaho Plateau, Nevada and Central Montana; cluster Clark Fork-Lolo Pass, Selkirk-Cabinets, and Missions-Glacier Park; and Greater Yellowstone-Grand Teton by itself). There is no evidence for isolation by distance following a geographic pattern based on latitude and longitude (R^2 = 0.0001; P = 0.45). These results indicate that neighboring trees are as similar genetically as trees separated by large geographic distances.

Summarizing these data by seed source for the comprehensive genetics profile, the number of unique alleles among 16 loci, range from eight to 32, assuming two alleles present per locus. Among the top 10 rust-resistance entries the proportion of observed polymorphic loci P = 0.238 and the average number of alleles is 20 (Table 1). Among all samples P = 0.258 and the average number of alleles is also 20. The oldest known living whitebark pine specimen located at Railroad Ridge is homozygous for 13 of the loci scored (no detectable protein activity at the remaining three loci): 12 loci are homozygous for common alleles and one locus is homozygous for a rare allele.

Chloroplast DNA (cpDNA) Variation

Among 160 samples, 34 unique multilocus SSR genotypes were generated. High levels of genetic diversity are found in all samples (expected heterozygosity (H_e) = 0.516 and number of alleles (Na) = 6.7, Table 4). Zone means are less diverse though still high (H_e = 0.481, Na = 4.2). The zones containing the largest amount of diversity are Bitterroots-Idaho Plateau then Clark Fork-Lolo Pass. The Selkirk-Cabinets zone contains the lowest level of diversity compared to the other zones.

Of the total variation measured, very little is found among zones, indicating that the zones share a large degree of genetic similarity and genetic structure (as did the isozyme data). Analysis of Molecular Variance calculations show 97 percent of the total variation is within zones, while only 3 percent among zones. Genetic identity also indicates high degrees of similarity among zones (Table 5). Zones are differentiated more by the cpDNA SSR data than with the isozyme data. Similarity among zone pairs ranges from 81.6 percent (Nevada and Selkirk-Cabinets zones) to 98.4 percent (Clark Fork-Lolo Pass and Selkirk-Cabinets zones). A graphical representation of the similarity among zones using Principal Component Analysis highlights that the Nevada zone is strongly separated from the other zones, whereas the Central Montana and Bitterroots-Idaho Plateau zones share proximity (Figure 2b). The Selkirk-Cabinets zone is closest to Clark Fork-Lolo Pass, followed by Missions-Glacier Park, Central Montana and Bitterroots-Idaho Plateau, then the Greater-Yellowstone-Grand Teton zone. The percentage of variation explained by the first two coordinates is 77.0 percent (87.9 percent by the first three coordinates). A mid-point phenogram (Figure 3b) shows similar zonal relationships: the Selkirk-Cabinets, Clark Fork-Lolo Pass, and Missions-Glacier Park zones form a group; followed by Central Montana and Bitterroots-Idaho Plateau, Greater-Yellowstone-Grand Teton, then the Nevada zone.

When removing the *a priori* defined zone designations from the samples, Principal Component Analysis using all continuous variables (cpDNA SSR markers, latitude, longitude, and elevation) indicates that each principal component is explaining different characteristics of the structure of the data. Cluster analysis using these variables shows that there are some imperfect groupings by zone, with individuals from the Nevada zone being strongly associated as a group. Discriminant analysis has a 44 percent mean correct classification rate for zone designations when using the cpDNA SSR markers. By adding latitude, longitude, and elevation to the analysis, the mean correct classification rate increases to 84 percent, showing that cpDNA variation alone does not recreate *a priori* zone designations well.

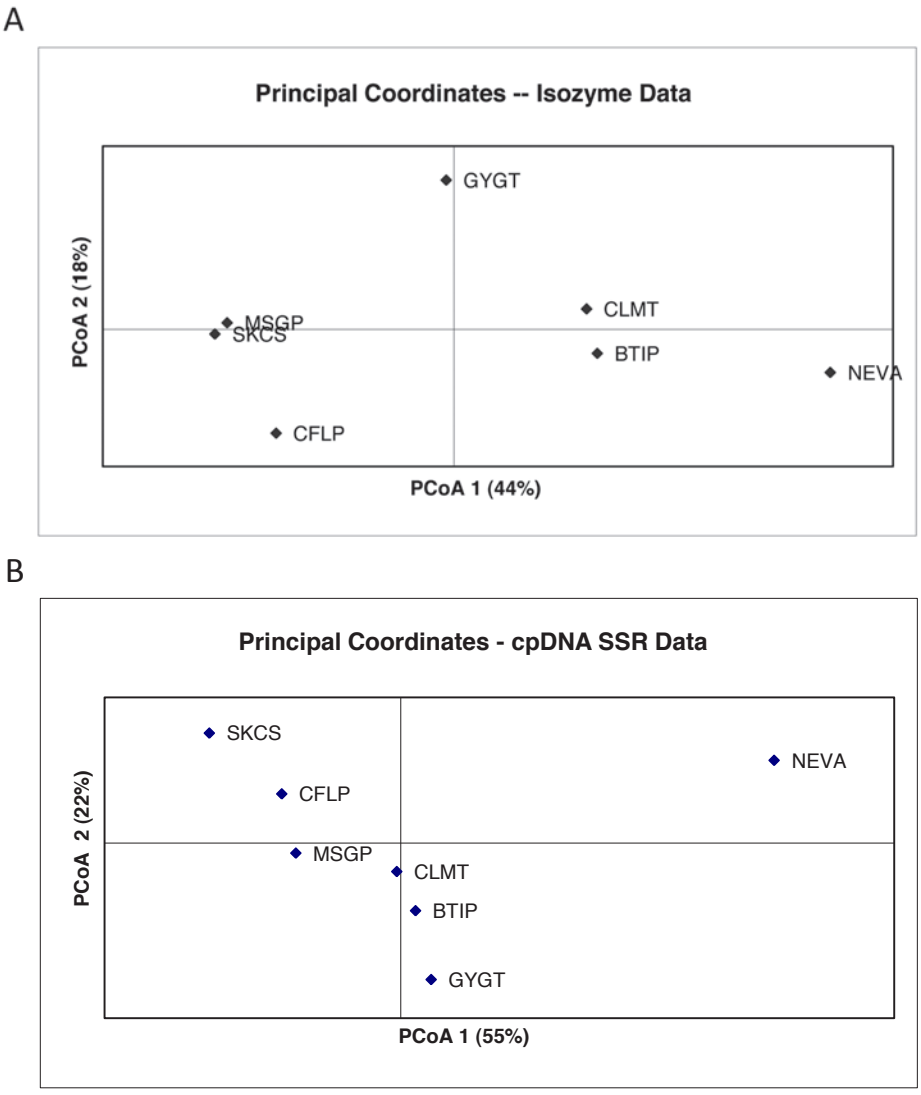

Figure 2. Principal Coordinate Analysis of seven zones of whitebark pine based on isozyme (A) and chloroplast (cpDNA) SSR markers (B). Zone designations as follows: BTIP=Bitterroots-Idaho Plateau, CFLP=Clark Fork-Lolo Pass, CLMT=Central Montana, GYGT=Greater Yellowstone-Grand Teton, MSGP=Missions-Glacier Park, NEVA=Nevada, SKCS=Selkirk-Cabinets.

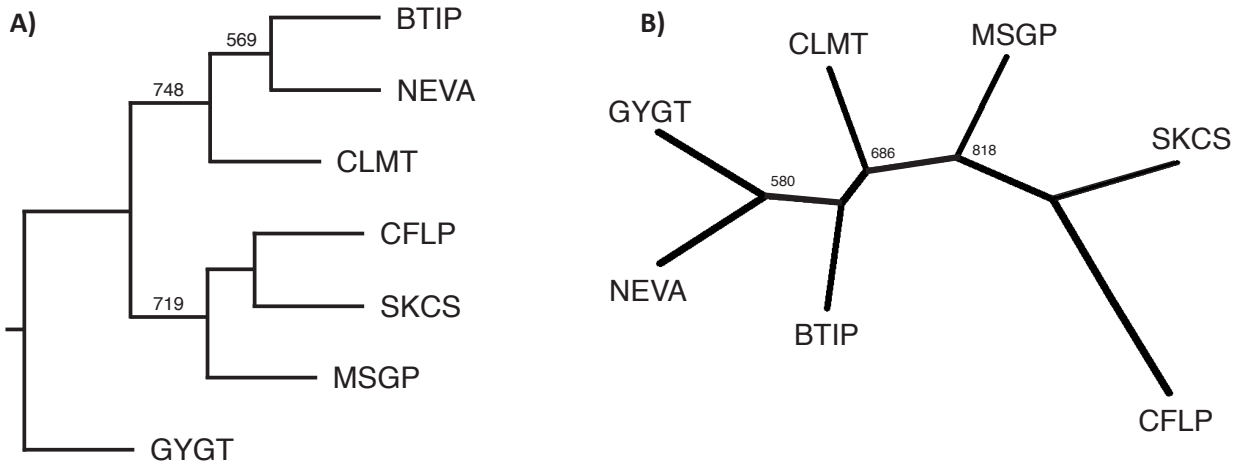

Figure 3. Mid-point phenograms of seven seed zones of whitebark pine from pairwise genetic distances using Neighbor-joining methods based on isozyme (A) and chloroplast (cpDNA) SSR markers (B). Significance of branches determined from 1,000 bootstrap replicates, resolved using Majority Rule extended method in Phylip v 3.68. Numbers at nodes represent the number of replicates out of 1,000 where the branch pattern occurred (>500). Zone designations as follows: BTIP=Bitterroots-Idaho Plateau, CFLP=Clark Fork-Lolo Pass, CLMT=Central Montana, GYGT=Greater Yellowstone-Grand Teton, MSGP=Missions-Glacier Park, NEVA=Nevada, SKCS=Selkirk-Cabinets.

Table 4. Genetic diversity statistics for Inland West whitebark pine samples based on three chloroplast (cpDNA) SSR markers. N = sample size; N_a = number of alleles; N_e = effective number of alleles; I = information index; H_e = expected heterozygosity.

Zone (Standard Errors)	Bitterroots-Idaho Plateau		Clark Fork-Lolo Pass		Central Montana		Greater Yellow-stone-Grand Teton		Missions-Glacier Park		Nevada		Selkirk-Cabinets		Zone Mean		All Samples	
N	22	(0.000)	22	(0.000)	18	(0.000)	29	(0.000)	25	(0.000)	21	(0.000)	23	(0.000)	22.9	(3.400)	160	(0.000)
Na	5	(1.155)	4.333	(1.333)	4.333	(2.333)	4.000	(1.000)	4.333	(1.453)	3.667	(0.882)	4.000	(1.528)	4.238	(0.480)	6.667	(1.764)
Na Freq. >= 5%	3.333	(0.667)	3.333	(0.882)	4.333	(2.333)	3.000	(1.000)	3.000	(1.000)	2.667	(0.333)	2.000	(0.577)	3.095	(0.648)	4.000	(1.528)
N_e	2.927	(1.025)	2.68	(0.997)	2.991	(1.745)	2.386	(0.841)	2.527	(0.960)	2.116	(0.431)	2.174	(0.807)	2.543	(0.395)	3.059	(1.460)
I	1.169	(0.310)	1.042	(0.341)	0.904	(0.555)	0.901	(0.400)	0.978	(0.367)	0.889	(0.249)	0.805	(0.399)	0.955	(0.096)	1.116	(0.434)
Number of Private Alleles	0.333	(0.333)	0.000	(0.000)	0.000	(0.000)	0.333	(0.333)	0.000	(0.000)	0.333	(0.333)	0.000	(0.000)	0.143	(0.178)	6.667	(1.764)
H_e	0.574	(0.124)	0.526	(0.139)	0.414	(0.216)	0.472	(0.162)	0.494	(0.149)	0.479	(0.120)	0.407	(0.188)	0.481	(0.035)	0.516	(0.172)

Table 5. Pairwise zone matrix of Nei's genetic identity for Inland West whitebark pine samples using three chloroplast (cpDNA) SSR markers.

Zone	Bitterroots-Idaho Plateau	Clark Fork-Lolo Pass	Central Montana	Greater Yellowstone-Grand Teton	Missions-Glacier Park	Nevada	Selkirk-Cabinets
Bitterroots-Idaho Plateau	1.000						
Clark Fork-Lolo Pass	0.958	1.000					
Central Montana	0.969	0.961	1.000				
Greater Yellowstone-Grand Teton	0.955	0.937	0.950	1.000			
Missions-Glacier Park	0.956	0.980	0.964	0.957	1.000		
Nevada	0.883	0.854	0.884	0.873	0.852	1.000	
Selkirk-Cabinets	0.921	0.984	0.937	0.899	0.966	0.816	1.000

Discussion

High levels of genetic diversity over all samples and within zones are found in whitebark pine when measured with isozymes. These measures of genetic diversity correspond to the species distribution occurring in eastern California, Idaho, Montana, Nevada, eastern Washington, and Wyoming (Figure 1, Table 1). Compared to whitebark pine from Oregon and Washington (USDA Forest Service 2009, unpublished report) these samples had higher mean expected heterozygosity (H_e = 0.271 vs. 0.194), more alleles per locus (Na = 4.0 vs. 1.9), and higher percent polymorphic loci (P = 100 vs. 65.0 percent). However, levels of diversity detected with chloroplast SSR variation in these two studies are similar for expected heterozygosity (H_e = 0.516 vs. 0.568) and effective number of alleles (Na = 3.1 in both studies).

Species with an outcrossing breeding system, large geographic range, long-life span, high fecundity, and wind-dispersed pollen and seeds, often have high levels of genetic diversity; though seed dispersal in whitebark pine is largely attributed to seed caching by Clark's nutcracker. Diversity levels in these collections of whitebark pine are similar to or greater than levels found in other conifers (Hamrick and others 1994, Steinhoff and others 1983, Wheeler and Guries 1982, Yang and Yeh 1993, Yeh and Layton 1979) that occupy the same forest cover type (Table 6). Expected heterozygosity is also greater, though similar in level to some other studies of whitebark pine (Bruederle and others 1998, Jørgensen and Hamrick 1997, Krakowski and others 2003, Rogers and others 1999, Yandell (1992) and limber pine (Hamrick and others 1994, Jørgensen and others 2002, Schuster and others 1989, Schuster and Mitton 2000) and similar to quaking aspen (H_e = 0.271), historically regarded as the most genetically diverse tree species (Cheliak and Dancik 1982). The degree of expected heterozygosity may be confounded due to the large geographic scale represented among the samples included in this study (38.08º to 48.98ºN latitude and -107.09º to -119.96ºW longitude).

Overall zones share a high degree of genetic similarity; however, some relationships appear to exist. The Nevada zone is differentiated from the other zones as seen by the unique maternal haplotype, high pairwise F_{ST} values, low overall genetic similarity values, and placement in the principal component plots, cluster diagrams, and phenograms. Though the three-year bumper cone crop cycle is predictable for members of the subgenus *Strobus*, another unique feature of whitebark pine from the Nevada zone is the timing of the three-year cycle; for example, recent bumper cone crops in the Northern Rockies were in 2003 and 2006, whereas bumper cone crops in Nevada followed in 2004 and 2007. The Greater Yellowstone-Grand Teton zone also shows a larger degree of differentiation from the other zones. The Selkirk-Cabinets, Clark Fork-Lolo Pass, and Missions-Glacier Park zones share similarity and cluster together in the principal component plots and phenograms, regardless of the genetic marker used for the analysis. The Central Montana and Bitterroots-Idaho Plateau zones also share similarity and cluster together in the principal component plots and cpDNA phenogram.

Table 6. Contrast of genetic diversity and *F*-statistics from isozyme date for selected members of the subgenus *Strobus* and associated species in the subalpine, mixed-conifer forest cover type. N = sample size (population or individuals); P = percent polymorphic loci; Na = number of alleles; H_e = expected heterozygosity; F_{ST}/ G_{ST} = genetic structure among populations or seed zones.

Species	# Loci	N	P	Na	H_e	F_{ST}/ G_{ST}	Citation
Strobus							
Pinus albicaulis	16	147	100	4	0.271	0.026	This study
	13	14	48.8	1.6	0.204	0.088	Yandell (1992)
	20	30	85	3	0.102	0.034	Jørgensen and Hamrick 1997
	19	9	79	2.1	0.154	0.025	Bruederle and others 1998
	21	80	54.5	-	-	0.004	Rogers and others 1999
	10	~510	70	2.0	0.262	0.061	Krakowski and others 2003
Pinus aristata	21	597	76	2.1	0.070	0.220	NFGEL, unpublished data
Pinus flexilis	12	5	93.3	2.4	0.295	0.035	Schuster and Mitton 2000
	20	30	95	3.7	0.186	0.101	Jørgensen and others 2002
	27	16	65.1	2.4	0.223	0.149	Hamrick and others 1994
	10	2	-	-	0.320	0.022	Schuster and others 1989
	23	550	59	2	0.165	0.147	NFGEL, unpublished data
Pinus monticola	12	28	92	2	0.191	0.148	Steinhoff and others 1983
Associated species in mixed-conifer type							
Abies lasiocarpa	25	10	56.5	2.49	0.181	0.109	Hamrick and others 1994
Picea engelmannii	26	19	63.5	2.53	0.182	0.101	Hamrick and others 1994
Pinus contorta ssp. *contorta*	21	66	12	-	0.180	0.076	Yang and Yeh 1993
	42	5	65	1.81	0.126	0.032	Wheeler and Guries 1982
	25	135	59	1.9	0.167	0.041	Yeh and Layton 1979

Gene flow in whitebark pine involves the movement of pollen and seed. Pollen dispersal (as elucidated by the cpDNA data) is not sufficient to swamp the genetic structure resulting from seed dispersal among seed zones (Figure 3a, 3b). Moreover, with such a small proportion of the variability found among seed zones, substituting bulked cone collections for individual-tree cone collections in the sampling scheme would likely not have shown any additional genetic structure. As with limber pine (Latta and Mitton 1997), the large difference between cpDNA and mtDNA data indicates pollen rather than seed movement is contributing to the bulk of gene flow in whitebark pine.

Whitebark pine is hypothesized to have inbred populations due to its discontinuous distribution, with isolated populations occurring at high elevations, and its seed dispersal by Clark's nutcracker. Supporting this, Jørgensen and Hamrick (1997) found a higher degree of inbreeding in whitebark pine than in other conifers, which they attributed to pollination within tree clusters. Here, mean outbreeding rates among seed sources within a zone as measured by isozymes indicate a lack of inbreeding, confirmed by neutral fixation index values (*F*), low inbreeding coefficient values (F_{IS} and F_{IT}), and high numbers of migrants (N_m = 9.354). Typical for conifers, N_m ranges from 5 to 20 (Ledig and others 1997, Mitton and Williams 2006). Limited pollen flow or seed dispersal near tree line, due to a climatic warming trend, might lead to subpopulation structuring, increases in inbreeding, or a reduction in genetic variation (Rogers and others 1999). Genetic structure (F_{ST}=0.026) among these 144 samples and 16 isozyme loci show a 6.5-fold increase in the fixation index as compared to three watersheds and 21 loci (F_{ST} = 0.004) sampled by Rogers and others (1999) in the eastern Sierra Nevada, but comparable in genetic structure (F_{ST} =0.025) among nine populations sampled in the Greater Yellowstone area (Bruederle and others 1998). Wind-pollination and seed caching by Clark's nutcrackers promotes a high degree of within-population variation in whitebark pine. Practical applications of these findings ratify existing management direction for cone collections used in restoration planting, where an operational lot of a wind-pollinated tree species contains seed from no fewer than 20 cone-bearing trees separated by 200 ft (61 m) in distance to minimize inbreeding depression in the subsequent progeny (USDA Forest Service 2010). Maintenance of three, bulked seed lots in inventory, for each seed zone and 400-foot (122 m) elevation band, satisfies the requirement of genetic sampling in space to maintain an effective population size of 60 individuals. Effective population size is defined as the number of breeding individuals in an idealized population that show the same amount of dispersion of allele frequencies under random genetic drift or the same amount of inbreeding as the population under consideration. Moreover, there is no need for additional seed orchard design considerations to maintain a suitable effective population size (spatially separating ramets of the same genotype by more than 80 feet (24 m)).

An opportunity to correlate these molecular data (F_{ST}) with quantitative trait data indices (Q_{ST}) observed in a common garden study (Mahalovich submitted), provides additional insights into the evolutionary forces operating in whitebark pine. The relative magnitude of these indices is informative regarding the role of natural selection and genetic drift as a causal agent of the observed degree of seed source differentiation in quantitative (adaptive) traits. Genetic drift

involves random fluctuations in the frequency of alleles that can lead to allele loss in isolated or small populations. Genetic drift can also occur when the size of a population is reduced through a genetic bottleneck. Present-day examples of potential bottlenecks affecting whitebark pine include mortality due to mountain pine beetle, uncontrolled fire, or blister rust. Omitting the Nevada samples, which were not available in the common garden study, F_{ST} is equal to 0.025. Q_{ST} in the control (uninoculated) seedlings for six-year height (0.12) and late winter cold hardiness (0.11) indicate differentiation due to directional selection and adaptation to local environments ($Q_{ST} > F_{ST}$), whereas survival (0.25) indicates differentiation due to genetic drift ($Q_{ST} = F_{ST}$). Q_{ST} in the treatment (inoculated) seedlings for spot symptoms per meter (0), no spotting symptoms (<0.01), shedding of infected needles (<0.01), and canker tolerance (0.02) indicate convergent selection favoring the same genotype in different environments. These traits however, are highly sensitive to the deliverable basidiospore load and could be more indicative of lighter spotting (the first observable symptom in needle tissue) in an artificial inoculation. Q_{ST} in the remaining treatment seedlings for survival (0.07), six-year height (0.19), late winter cold hardiness (0.10), percent rust resistance (0.14), and the individual, rust-resistance traits of early stem symptoms (0.03), fungicidal short shoot (0.17), and bark reaction (0.37) favor differentiation due to directional selection and adaptation to local environments. Where $Q_{ST} > F_{ST}$ and the average observed heterozygosity in the top 10 rust resistant selections is less than all samples ($0.238 < H_o < 0.258$, Table 1), the introduction of blister rust around 1925 in whitebark pine cover types (McDonald and Hoff 2001) appears to be exerting selection pressure and adaptation to local environments in the last two generations. The blister rust introduction site corresponds to the geographic location of the Selkirk-Cabinet zone, which also exhibits the lowest level of genetic diversity ($H_e = 0.407$, Table 4). These data however, do not indicate there is a strong enough selection pressure to begin to identify unusual genes limited in their geographic distribution.

Mahalovich (submitted) supports the consolidation of six seed zones to four in the Northern Rockies. The current study evaluating neutral markers is largely in agreement with those findings and further supports consolidation of six seed zones to *three* in the Northern Rockies, while maintaining the uniqueness of the Nevada zone. Nei's genetic identity supports combining the Clark Fork-Lolo Pass and Missions-Glacier Park zones. The Principal Component Analysis (PCA) for the isozyme data extends the similarity among the Clark Fork-Lolo Pass, Missions-Glacier Park and Selkirk-Cabinets zones. Though the PCA shows similarity among the Bitterroots-Idaho Plateau, Central Montana and Nevada zones, samples from Nevada zone were not available for the common garden study. The Nevada samples reported here all contain maternal haplotype 2; therefore, the Nevada zone will remain a distinct seed zone. Isozyme data would then support combining the Bitterroots and Central Montana zones. Owing that more samples were not available south of 44.5° N latitude (from central and southern Idaho) and that the common garden study emphasizes traits with adaptive value, these two zones will remain distinct. Genetic diversity statistics using the three chloroplast SSR markers also show the Bitterroots-Idaho Plateau zone to be distinct with the highest level of genetic diversity. Lastly, the four adaptive traits from the common garden study, isozyme data and three chloroplast SSR markers reported here, all support the Greater Yellowstone-Grand Teton zone remaining a distinct zone. Taken collectively, the Bitterroots-Idaho Plateau, Central Montana, and Greater Yellowstone-Grand Teton zone boundaries remain as originally defined (Mahalovich submitted); however, the Selkirk-Cabinets, Clark Fork-Lolo Pass and Missions-Glacier Park zones show sufficient overlap to support consolidation into one zone, renamed the 'Inland Northwest'. This consolidated zone is approximate to Bailey's M333 Northern Rocky Mountain Forest-Steppe—Coniferous Forest—Alpine Meadow Province (Bailey 1995). The geographic areas defined by the three remaining Northern Rockies zones show little congruence to other mountain province boundaries in the temperate steppe division.

Since both studies incorporate genetic data, these realigned seed zones will also serve as breeding zones or geographic areas based on the anticipated adaptability of an improved population of trees in the genetic restoration program for the Inland West (Mahalovich and Dickerson 2004). These five breeding zones are already in effect for seed procurement planning, genetic testing and seed orchard establishment. Field data (10 years from seed) on control (uninoculated) and treatment (inoculated) seedlings planted in a long-term field test are being evaluated in combination with climatic data to reconfirm the veracity of the zone boundaries.

The development of a comprehensive genetic profile (Table 1) to determine those seed sources at risk for being lost due to their apparent uniqueness will facilitate gene conservation in whitebark pine. *Ex situ* conservation activities in the Inland West Genetic Restoration Program (Mahalovich 2000, Mahalovich and Dickerson 2004) include establishing seed orchards, clone banks, and long-term genetic tests, while building long-term seed and pollen banks. *In situ* conservation is being met by: (1) federal lands classified in wilderness areas, (2) Research Natural Areas (RNAs) specific to the USDA Forest Service (Evenden and others 2001), and (3) the network of plus trees designated and protected against mountain pine beetle across multiple federal ownerships (USDA Forest Service, USDI-Bureau of Land Management and USDI-National Park Service). Upwards of 950 plus trees are designated in the program. A recent assessment shows 21 percent of those plus trees have been lost to mountain pine beetle, fire, and blister rust in descending order.

For the USDA Forest Service Northern Region there are 25 RNAs with a whitebark pine component covering 53,771 acres, and 23 RNAs covering 34,416 acres in the Intermountain Region (Evenden and others 2001). Identification of potential candidate areas involves the selection of seed sources utilizing the available genetic statistics

(Table 1). Among the 48 RNAs there is little geographic representation from the Clark Fork-Lolo Pass and Missions-Glacier Park zones. Using the Missions-Glacier Park zone as an example, the sample with the highest number of alleles (n=25) and proportion of polymorphic loci (P = 0.563) is Hornet Mountain (Flathead National Forest), whereas Big Mountain from the same zone qualifies as a candidate area because it has the highest percent rust resistance. Blacklead Mountain (Clearwater National Forest) from the Clark Fork-Lolo Pass zone qualifies as a candidate area with high proportion of polymorphic loci (P = 0.438) and a highly desirable combination of blister rust resistance *and* late winter cold hardiness. Mahalovich (submitted) characterized blister rust resistance in whitebark pine from the Northern Rockies as having an unfavorable correlation to late winter cold hardiness. The sample with the lowest number of unique alleles (n=13) is from Railroad Ridge (Bitterroots-Idaho Plateau zone), with no polymorphic loci (P = 0). Railroad Ridge at first glance may not receive additional consideration; however, other factors such as containing a rare allele and supporting the oldest specimen of whitebark pine may elevate it as a candidate area for further evaluation. Where RNAs embody preservation, other areas (for example, Corbly Gulch, Gallatin National Forest, Central Montana zone, P = 0.5 and n=24,) may facilitate active management favoring silvicultural prescriptions to minimize species encroachment or to prepare seedbeds for natural regeneration.

Collectively these data show complementary relationships between molecular markers and adaptive traits in a common garden study. Continued analysis of long-term field data, incorporating climatic variables, and artificial inoculations of additional cone collections will facilitate the identification of neutral markers influenced by selection and genetic drift, particularly those genotypes demonstrating resistance to blister rust and tolerance to mountain pine beetle. A comprehensive genetics profile will also benefit future research addressing the molecular basis of blister rust resistance by facilitating the identification of seed sources with both blister rust resistance and non-segregating isozyme loci. Moreover, there is sufficient genetic diversity and genetic variation to support the continuation of a rust resistance screening and genetic restoration program for this species.

Management Implications (USDA Forest Service 2010)

Operational cone collections in the field shall be comprised of no fewer than 20 cone-bearing trees separated by 200 ft (61 m) in distance.

For each seed zone and 400-foot (122 m) elevation band, a minimum of three, bulked seedlots shall be maintained to achieve an effective population size of 60 individuals at any given time.

Seed zones in the Whitebark Pine Inland West Genetic Restoration Program have been consolidated from seven to five geographic areas. The revised seed zones shall be used for seed procurement planning and seed transfer.

These five seed zones also serve as breeding zones for seed orchard design, future genetic testing and breeding orchard establishment.

Seed orchard design will adhere to considerations for wind-pollinated conifer species with no increase in spatially separating ramets of a genotype by more than 80 feet (24 m)).

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References

Bailey, Robert G. 1995. Description of the ecoregions of the United States. 2d ed. rev. and expanded (1st ed. 1980) Misc. Publ. No. 1391 (rev.), Washington, DC: USDA Forest Service. 108 p. with separate map at 1:7,500,000.

Bruederle, Leo P.; Tomback, Diana F.; Kelly, Kathleen K.; Hardwick, Renea C. 1998. Population genetic structure in a bird-dispersed pine, *Pinus albicaulis* (Pinaceae). Canadian Journal of Botany. 76: 83-90.

Burr, Karen E.; Eramian, Aram; Eggleston, Kent. 2001. Growing whitebark pine seedlings for restoration. In: Tomback, Diana F.; Arno, Steven F.; Keane, Robert E., eds. Whitebark pine communities: ecology and restoration. Washington, DC: Island Press. 325-345.

Cheliak, William M.; Dancik, Bruce P. 1982. Genic diversity of natural populations of a clone forming tree *Populus tremuloides*. Canadian Journal of Genetics and Cytology. 24: 611-616.

Evenden, Angela G.; Moeur, Melinda; Shelly, J. Stephen; Kimball, Shannon F.; Wellner, Charles A. 2001. Research natural areas on National Forest System lands in Idaho, Montana, Nevada, Utah, and western Wyoming: A guidebook for scientists, managers, and educators. General Technical Report RMRS-GTR-69. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. 84 p.

Felsenstein, Joseph. 2005. PHYLIP (Phylogeny Inference Package) version 3.6. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle.

Hamrick, James L.; Schnabel, Andrew F.; Wells, Pat V. 1994. Distribution of genetic diversity within and among populations of Great Basin Conifers. In Natural History of the Colorado Plateau and Great Basin, K. T. Harper, L. L. St. Clair, K.H. Thorne, and W.M. Hess (eds). University of Colorado Press, p 147-161.

Jørgensen, Stacy M.; Hamrick, James L.; Wells, P. V. 2002. Regional patterns of genetic diversity in *Pinus flexilis* (Pinaceae) reveal complex species history. American Journal of Botany. 89(5): 792-800.

Jørgensen, Stacy M.; Hamrick, James L. 1997. Biogeography and population genetics of whitebark pine, *Pinus albicaulis*. Canadian Journal of Forest Research. 27: 1574-1585.

Krakowski, Jodie; Aitken, Sally N.; El-Kassaby, Yousry A. 2003. Inbreeding and conservation genetics in whitebark pine. Conservation Genetics. 4: 581-593.

Latta, Robert G.; Mitton, Jeffrey B. 1997. A comparison of population differentiation across four classes of gene marker in limber pine (*Pinus flexilis* James). Genetics. 146: 1153-1163.

Ledig, F. Thomas; Jacob-Cervantes, Virginia; Hodgskiss, Paul D.; Eguiluz-Piedra, Teobaldo. 1997. Recent evolution and divergence among populations of a rare Mexican endemic, Chihuahua spruce, following Holocene climatic warming. Evolution. 51(6): 1815-1827.

Lewis, Paul O.; Zaykin, Dmitri. 2001. Genetic Data Analysis: Computer program for the analysis of allelic data. Version 1.0(d16c). Free program distributed by the authors over the internet from http://lewis.eeb.uconn.edu/lewishome/software.html.

Little, Elbert L. 1971. Atlas of the United States Trees. Volume 1 Conifers and important hardwoods. USDA Forest Service, Miscellaneous Publication No. 1146.

Mahalovich, Mary F. [Submitted May 2009]. Genetic differentiation of *Pinus albicaulis* Engelm. populations from the Northern Rocky Mountains. The Americas Journal of Plant Science and Biotechnology.

Mahalovich, Mary F.; Burr, Karen E.; Foushee, David L. 2006. Whitebark pine germination, rust resistance and cold hardiness among seed sources in the Inland Northwest: Planting Strategies for Restoration. In: National Proceedings: Forest and Conservation Nursery Association; 2005 July 18-20; Park City, UT, USA. Proceedings RMRS-P-43. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station: 91-101.

Mahalovich, Mary F.; Dickerson, Gary A. 2004. Whitebark pine genetic restoration program for the Intermountain West (United States). In: Sniezko, R. A., Samman, S.; Schlarbaum, S. E.; Scott, E.; Kriebel, H. B., eds. Breeding and genetic resources of five-needle pines: growth, adaptability and pest resistance. 2001 July 23-27; Medford, OR, USA. IUFRO Working Party 2.02.15 Proceedings RMRS-P-32. Fort Collins, CO: USDA Forest Service, Rocky Mountain Research Station: 181-187.

Mahalovich, Mary F. 2000. Whitebark Pine Restoration Strategy—Some genetic considerations. In: Nutcracker Notes, January 1, 2000. Number 11. USDA Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory. Missoula, MT. 4 p.

McDonald, Geral I.; Hoff, Raymond. 2001. Blister rust: an introduced plague. In: Whitebark pine communities, ecology and restoration. Tomback, Diana F.; Arno, Steve F.; Keane, Robert E. (eds.). Island Press, Washington, DC. 193-220.

McLaughlin, Marie. 2010. [Personal communication]. June 15, Coeur d'Alene, ID: U.S. Department of Agriculture, Forest Service, Northern Region, Coeur d'Alene Nursery.

Mitton, Jeffrey B.; Williams, Claire G. 2006. Geneflow in conifers. In: Landscapes, Genomics, and Transgenic Conifers (Claire G. Williams, ed). Springer. 147-168.

Mitton, Jeffrey B. 1995. Genetics and physiological ecology. In: Smith, William K.; Hinckley, Thomas M., eds. Ecophysiology of coniferous forests. San Diego, CA: Academic Press, Inc.: 1-36.

Nei, Masatoshi. 1972. Genetic distance between populations. American Naturalist. 106: 283-292.

Peakall, Rodney; Smouse, Peter E. 2005. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes. 6: 288-295.

Perkins, Dana L.; Swetnam, Thomas W. 1996. A dendroecological assessment of whitebark pine in the Sawtooth-Salmon River region, Idaho. Canadian Journal Forest Research. 26: 2123-2133.

Richardson, Bryce A. 2001. Gene flow and genetic structure of whitebark pine (*Pinus albicaulis*): Inferences into bird-dispersed seed movement and biogeography. Unpublished Master Thesis. University of Idaho. 55 p.

Richardson, Bryce A.; Klopfenstein, Ned B.; Brunsfeld, Steven J. 2002. Assessing Clark's nutcracker seed-caching flights using maternally inherited mitochondrial DNA of whitebark pine. Canadian Journal of Forest Research. 32: 1103-1107.

Rogers, Deborah L.; Millar, Constance I.; Westfall, Robert D. 1999. Fine-scale genetic structure of whitebark pine (*Pinus albicaulis*): assocations with watershed and growth form. Evolution. 53(1): 74-90.

Schuster, William S. F.; Mitton, Jeffrey B. 2000. Paternity and gene dispersal in limber pine (*Pinus flexilis* James). Heredity. 84: 348–361.

Schuster, William S. F.; Alles, David L., Mitton, Jeffrey B. 1989. Gene flow in limber pine: evidence from pollination phenology and genetic differentiation along an elevational transect. American Journal of Botany. 76(9): 1395-1403.

Spitze, Ken. 1993. Population structure in *Daphnia obtuse*: Quantitative genetic and allozymic variation. Genetics 135: 367-374.

Steinhoff, Raymond J.; Joyce, Dennis G.; Fins, Lauren. 1983. Isozyme variation in *Pinus monticola*. Canadian Journal of Forest Research. 13: 1122-1131.

USDA Forest Service. 2010. Combined Seed Handbook FSH 2409.26f, Northern, Rocky Mountain, Southwestern and Intermoutain Regions. Missoula, MT, Lakewood, CO, Albuquerque, NM and Ogden, UT.

USDA Forest Service. 2009. Assessment of Genetic Diversity of Whitebark Pine in Washington and Oregon. National Forest Genetics Laboratory Lab Report #156, 157, and 221. NFGEL, Placerville, CA.

USDA Forest Service. 2003. National Forest Genetic Electrophoresis Laboratory Standard Operating Procedures. NFGEL, Placerville, CA.

Vendramin, Giovanni G.; Lelle, L.; Rossi, P.; Morgante, Michele. 1996. A set of primers for the amplification of 20 chloroplast microsatellites in Pinaceae. Molecular Ecology. 5: 595-598.

Wheeler, Nicholas C.; Guries, Raymond P. 1982. Population structure, genetic diversity and morphological variation in *Pinus contorta* Dougl. Canadian Journal of Forest Research. 12: 595-606.

White, Tim L.; Adams, W. Thomas; Neale, David B. 2007. Forest Genetics. CABI Publishing, Cambridge, MA. 682 p.

Wright, Sewell. 1951. The genetical structure of populations. Annals of Eugenics. 15: 323-354.

Wu, Junyuan; Krutovskii, Konstantin V.; Strauss, Steven H. 1998. Abundant mitochondrial genome diversity, population differentiation and convergent evolution in Pines. Genetics. 150: 1605-1614.

Yandell, Ulla G. 1992. An allozyme anlayis of whitebark pine (*Pinus albicaulis* Engelm). Unpublished master's thesis. University of Nevada, Reno. 64 p.

Yang, Rong-Cai; Yeh, Francis C. 1993. Multilocus structure in *Pinus contorta* Dougl. Theoretical and Applied Genetics. 87: 568-576.

Yeh, Francis C.; Yang, Rong-Cai; Boyle, Timothy. 1997. Popgene Version 1.32. University of Alberta, Edmonton, Alberta.

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