

GENIC DIVERSITY, GENETIC STRUCTURE, AND MATING SYSTEM OF BREWER SPRUCE (PINACEAE), A RELICT OF THE ARCTO-TERTIARY FOREST¹

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Brewer spruce (*Picea breweriana*), a relict of the widespread Arcto-Tertiary forests, is now restricted to a highly fragmented range in the Klamath Region of California and Oregon. Expected heterozygosity for 26 isozyme loci, averaged over 10 populations, was 0.121. More notable than the relatively high level of diversity when compared to other woody endemics was the strong decrease in expected heterozygosity with latitude. Differentiation (F_{ST}) was 0.152, higher than values for many north temperate conifers with larger distributions. The number of migrants per generation (Nm) was 1.34 or 2.70, depending on the method of estimation. Inbreeding appeared low; F_{IS} was only 0.003, in agreement with multilocus population outcrossing rates (t_m), which were generally well above 0.90. No difference in t_m was found between isolated vs. clustered trees. However, the number of seeds per cone was greatest in the densest populations; t_m is a measure of effective outcrossing after mortality in the embryonic stage, whereas a reduced number of seeds per cone indicates self pollination. Selfing increased after logging; outcrossing rate before logging was 0.961 and after logging, 0.756. Despite Brewer spruce's narrow, fragmented distribution, the outlook for its conservation was good, with the exception of possible negative effects of logging.

Key words: fragmentation; genetic structure; heterozygosity; isozymes; relict species; selfing.

Brewer spruce (*Picea breweriana* Wats.) is a relict of the Arcto-Tertiary forest, most species of which are now extinct in western North America. According to the fossil record, Brewer spruce had a wide distribution in the Pliocene and Miocene, at least as far east as Idaho and Nevada, north to central Oregon, and south to central California (Wolfe, 1964). The fossil species, Sonoma spruce (*Picea sonomensis* Axelrod), which is synonymous with Brewer spruce, occurred in the Creede Flora in the San Juan Mountains of southwestern Colorado in the Oligocene (Axelrod, 1987).

The Arcto-Tertiary flora was reduced by increasingly dry climates in the interior West, especially as mountain building accelerated at the close of the Miocene. Cool moist forests shrunk toward the coast and higher elevations (Whittaker, 1961). Brewer spruce is now endemic to the Klamath Geomorphological Province, which retains forests most nearly equivalent to the western North American Arcto-Tertiary forests (Whittaker, 1960; Sawyer and Thornburgh, 1977). Boundaries of the Klamath Province are defined in different ways depending on whether they are based on a strict geomorphological interpretation or weighted by ecological considerations (Fig. 1). The region generally includes the Siskiyou Mountains, Salmon Mountains, Marble Mountains, North Yolla Bolly Mountains, Scott Bar Mountains, and Trinity Alps, an area of about 50 300 km² (Miles and Gouday, 1997). The substrate is a complex of Paleozoic and Mesozoic formations distinct from the surrounding younger rocks of the Coast Ranges, Cascade Range, and Sacramento Valley (Whittaker, 1960; Sawyer and Thornburgh, 1977).

The International Union for Conservation of Nature and Natural Resources (IUCN) declared the Klamath an "Area of Global Botanical Significance," one of only seven in North America (Villa-Lobos, 2003). Regions surrounding the Klamaths were submerged at various periods in their paleohistory, but the Klamaths escaped submergence. Neither were they extensively glaciated nor subject to the volcanism of the Cascade Range to the east (Whittaker, 1961; Irwin, 1966). Neighboring floras were decimated while the Klamath Geological Province, relatively stable for at least 100 million years (Myr), became a refuge for many species; i.e., about 3500 plants, of which 280 are endemic (Villa-Lobos, 2003).

The Klamath Province is a meeting ground for species that are at the southern limit of their range and others that are at their northern limit, as well as species from the Cascades and the Sierra Nevada. The rugged terrain and complex geology and soils contribute to species richness (Whittaker, 1960). Patches of serpentine soil, in particular, harbor many plants rare outside the Klamath region and may have provided stepping stones for Brewer spruce and the Klamath flora to migrate across the topography in response to climatic alterations (R. H. Waring, Oregon State University, personal communication). Although the regional climate is Mediterranean, a strong moisture and temperature gradient exists from the coast inland (Whittaker, 1960; Waring, 1969). Brewer spruce is excluded from the dryer, eastern portion of the Klamath Province because it is adapted to cool temperatures during the growing season and closes its stomata when evaporative demand is high, which puts it at a disadvantage against competitors (Waring et al., 1975).

Brewer spruce covers a northwest–southeast range of 228 km from Iron Mountain, Oregon, to East Weaver Lake, California, nearly the entire north–south extent of the Klamath Province (Fig. 1). It occurs within 22 km of the Pacific Coast (Waring et al., 1975), and an isolate near Castle Crags, California (Haddock, 1938), is about 145 km inland. The north-

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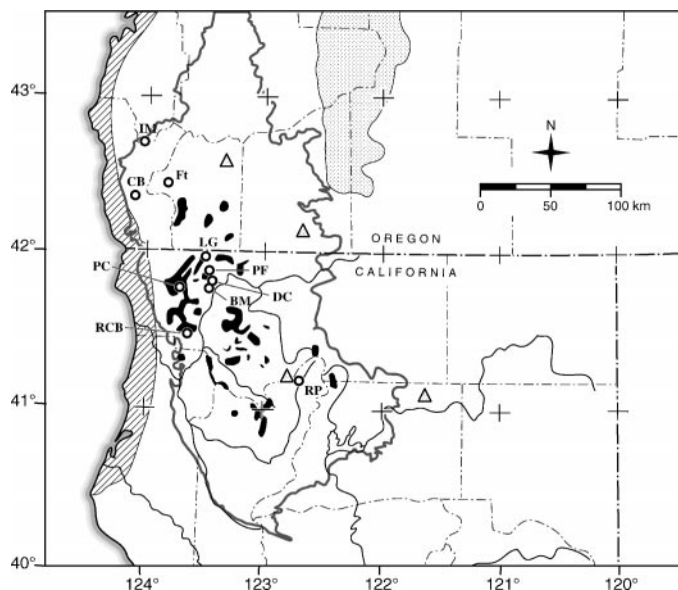


Fig. 1. The range of Brewer spruce (in black) after Griffin and Critchfield (1972) and Waring et al. (1975), adjoining ranges of Sitka spruce (cross-hatching) and Engelmann spruce (triangles and stippling) after Little (1971), the locations of Brewer spruce populations (open circles) sampled for isozyme analysis, and boundaries of the Klamath-Siskiyou region in Oregon after DellaSala et al. (1999) and in California after Miles and Gouday (1997). IM = Iron Mountain; Ft = Flat top; CB = Collier Butte; LG = Little Grayback; PF = Poker Flat; DC = Doolittle Creek; PC = Prescott Cabin; BM = Baldy Mountain; RCB = Rock Creek Butte; RP = Russian Peak.

ernmost population, at Iron Mountain, may be isolated from other Brewer spruce by as much as 29 km. The species ranges in elevation from 560 to 2300 m a.s.l. (Waring et al., 1975). It is thin-barked and susceptible to fire (Thornburgh, 1990), so it often occurs in relatively open stands on rocky and infertile soils, which are less prone to hot fires. Brewer spruce is notable for its long, delicate, pendulous branches, which gives it its other common name, weeping spruce.

Brewer spruce is associated with at least 15 other conifers and 21 broadleaf evergreen trees and shrubs (Waring et al., 1975). Two of its most common associates are Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) and Shasta red fir (*Abies magnifica* A. Murr. var. *shastensis* Lemmon). These and other associates attain greater height and older ages, so Brewer spruce is often found in the subcanopy. Maximum height, diameter at breast height (dbh), and age of Brewer spruce are 54 m, 162 cm, and 900 yr, respectively (Waring et al., 1975; Cowley, 2000).

An isolate of Engelmann spruce (*Picea engelmannii* Parry ex Engelm.) is sympatric with Brewer spruce on the slopes of Russian Peak (Sawyer and Thornburgh, 1969, 1970). Another spruce, Sitka spruce [*Picea sitchensis* (Bong.) Carr.], occurs along the Pacific Coast within 18.5 km of the nearest Brewer spruce but much lower in elevation (Waring et al., 1975). Brewer spruce is generally found between 560–2300 m a.s.l., while Sitka spruce in the Klamath Region is not found more than 200 m a.s.l. (Hickman, 1993). In an ecological reconnaissance, no hybrids between Brewer spruce and either Engelmann or Sitka spruce were observed (Sawyer and Thornburgh, 1969). In fact, repeated attempts at controlled pollination have failed to produce hybrids between Brewer spruce and other spruce species (Gordon, 1986), and phylogenies

based on molecular markers suggest that Brewer spruce may stand alone in the genus with no close relatives (Wellendorf and Simonsen, 1979; Sigurgeirsson and Szmidt, 1993; Ledig et al., 2004; C. S. Campbell, University of Maine, personal communication). The presence of alkaloids from two distinct biosynthetic pathways, one of which is, so far, novel for conifers, underscores the uniqueness of the species (Schneider et al., 1995).

Within its present range, Brewer spruce occurs singly or in small disjunct populations (Thornburgh, 1990). Thornburgh (1990) thought that low density, isolation, the overlapping distribution of male and female strobili within the crown, and their concurrent development encouraged selfing. Certainly, other relict spruces with fragmented ranges and small populations have mixed mating systems characterized by rates of selfing unusually high for conifers (Ledig et al., 1997, 2000, 2002). Selfing or close inbreeding reduces seed production (Franklin, 1970), reducing population viability in typically outbreeding conifer populations.

Brewer spruce is not protected from logging or disturbance, and projected climate change may adversely affect it and other montane and subalpine species in the Klamath Mountains. The Klamath peaks at only about 2750 m a.s.l., thus limiting the opportunity for montane species to migrate higher in elevation. Therefore, it is important for the conservation of Brewer spruce to know whether it has lost genetic diversity that might restrict its ability to respond to environmental changes, and whether like other rare spruces, it also experiences high levels of selfing, which could lead to reproductive failure.

Areas high in genetic diversity are likely to represent populations best able to cope with change and, therefore, are the best areas to focus conservation efforts. The structure of diversity should inform decisions on whether few or many reserves should be managed and monitored. Mating system estimates indicate whether inbreeding and subsequent depression of fitness may be a problem. Therefore, we undertook a survey to determine the level and pattern of genetic diversity in Brewer spruce and estimate mating system parameters. Because of its relictual status, and based on studies of Mexican spruces that occur in small, fragmented populations (Ledig et al., 1997, 2000, 2002), we hypothesized that Brewer spruce would have low levels of diversity within populations and high levels of differentiation among populations. We further hypothesized that it would have a mixed mating system with substantial selfing. Our sample also provided the opportunity to compare mating systems before and after selective timber harvest and in relatively isolated trees vs. those with many near neighbors.

MATERIALS AND METHODS

USDA Forest Service personnel from the Pacific Southwest Region's North Zone Tree Improvement Center collected cones from nine populations of Brewer spruce, and the staff of the Gold Beach Ranger District, Siskiyou National Forest, collected cones from one other population, Collier Butte (Fig. 1). Most populations were sampled in the 1985 cone year. We intended to sample 20 trees in each population. However, at Iron Mountain, only 10 trees were initially sampled; therefore, a second cone collection was made from 32 trees in 1999. Cones were collected at Baldy Mountain in 1989. At Flat top, 11 trees in the sample were collected in 1981 by the staff of the USDA Forest Service Pacific Northwest Region's Dorena Tree Improvement Center, and the remaining nine trees in the sample were collected in 1985 by the staff of the Pacific Southwest Region's North Zone Tree Improvement Center.

To meet the goal of at least 20 cone-bearing trees, it was often necessary

to extend samples over several kilometers. The populations and their characteristics relevant to the genetic analysis are as follows:

Iron Mountain—(Powers Ranger District, Siskiyou National Forest; 42°41' N, 124°09' W, 1190 m a.s.l.)—This, the northernmost population of Brewer spruce, occupies the peak of Iron Mountain, which is about 22 km from the coast (Shea, 1996). Iron Mountain is actually in the Rogue River Mountains, immediately north of the Rogue River, which is often accepted as the northern boundary of the Klamath Region. The population of Brewer spruce at Iron Mountain is the most compact of the populations sampled. The sampled trees were separated by no more than 100 m and were the canopy dominants. The vegetation on the ridge and the slopes surrounding the summit is floristically rich and includes eight other conifers (Baker, 1956). The Brewer spruce are small, but fairly old; Shea (1996) reported increment cores for three trees that ranged in age from over 180 to over 400 yr. The oldest tree aged was only 12 m tall and 43.7 cm dbh.

Flattop—(Galice Ranger District, Siskiyou National Forest; 42°26' N, 123°48' W, 1340 m a.s.l.)—The terrain is relatively level, in contrast to the north slopes on which Brewer spruce usually occurs, and the soils are ultramafic. Cones were collected from 11 trees immediately prior to selective timber harvest and from a different set of nine trees 4 years later after logging. The trees sampled were scattered over at least 2.8 km and 130 m in elevation. Although many Brewer spruce were cut, Brewer spruce probably represented a higher proportion of the stand after logging than before harvest. Prior to logging, the Brewer spruce were associated with larger dominants, true fir (*Abies* spp.), sugar pine (*Pinus lambertiana* Dougl.), and Jeffrey pine (*Pinus jeffreyi* Grev. and Balf.). After logging, the stand was very open and the spruces were fully exposed to sunlight. Fifteen years later, when we revisited the stand, many Brewer spruce were infested with dwarf mistletoe (*Arceuthobium campylopodum* Engelm.) and were dying or dead. No cones were produced on heavily infected trees.

Collier Butte—(Gold Beach Ranger District, Siskiyou National Forest; 42°22' N, 124°08' W, 1070 m a.s.l.)—The stand was compact and fairly dense, with less than 500 m between the most distant trees in the sample and little change in elevation. Brewer spruce were associated with Douglas-fir, Sadler oak (*Quercus sadleriana* R. Br.), rhododendron (*Rhododendron* sp.), and manzanita (*Ceanothus* sp.).

Little Grayback—(Happy Camp Ranger District, Klamath National Forest; 41°59' N, 123°31' W, 1330 m a.s.l.)—Brewer spruce makes its best development at Little Grayback (Waring et al., 1975), where it grows under a canopy of Shasta red fir, white fir [*Abies concolor* (Gord. and Glend.) Hildeb.,], and Douglas-fir. The stand is dense, and sample trees spanned only about 500 m and a range in elevation of 60 m. The trees were among the largest sampled, with many over 30 m tall and 75 cm dbh. Reproduction was good.

Poker Flat—(Happy Camp Ranger District, Klamath National Forest; 41°56' N, 123°32' W, 1510 m a.s.l.)—Samples were widely distributed over at least 2.4 km and 180 m in elevation. Some trees were very isolated, with no other Brewer spruce in sight. Much of the area has been selectively cut, leaving the smaller spruce, although one tree was over 30 m tall and about 130 cm dbh.

Doolittle Creek—(Happy Camp Ranger District, Klamath National Forest; 41°50' N, 123°28' W, 1150 m a.s.l.)—Samples were distributed over about 1.2 km and 90 m based on global positioning system (GPS) readings in 2000. The area is floristically rich, and associates include white fir, incense-cedar (*Libocedrus decurrens* Torr.), Port-Orford-cedar (*Chamaecyparis lawsoniana* [A. Murr.] Parl.), Douglas-fir, and Pacific yew (*Taxus brevifolia* Nutt.). The stand has been selectively logged, and dwarf mistletoe is common on the spruces.

Prescott Cabin—(Gasquet Ranger District, Six Rivers National Forest; 41°49' N, 123°44' W, 1260 m a.s.l.)—Samples were widely distributed over

about 4.25 km, based on GPS, and 120 m in elevation. Some trees in the sample were quite isolated from other Brewer spruce, and some grew in dense clusters on rocky, north slopes. Reproduction was good.

Baldy Mountain—(Happy Camp Ranger District, Klamath National Forest; 41°48' N, 123°28' W, 1580 m a.s.l.)—Samples were distributed over a total of about 1.5 km. However, 17 of the 20 trees in the sample were within 300 m of each other in a fairly dense stand. Conifer associates included white fir, Shasta red fir, Douglas-fir, incense-cedar, sugar pine, western white pine (*Pinus monticola* Dougl. ex D. Don), and Pacific yew.

Rock Creek Butte—(Ukonom Ranger District, Klamath National Forest; 41°33' N, 123°39' W, 1300 m a.s.l.)—Samples were distributed in clusters over about 2 km and 275 m in elevation. Some trees exceeded 35 m in height and 85 cm dbh. Reproduction was prolific.

Russian Peak—(Scott River Ranger District, Klamath National Forest; 41°20' N, 122°56' W, 1420 m a.s.l.)—This is the only area where Brewer spruce is sympatric with a congener, Engelmann spruce. The area had been selectively logged but is now protected in the Russian Peak Wilderness Area. The samples were distributed over 550 m of elevation and the most distant trees sampled were nearly 5 km apart.

Except for Flattop, stands occurred on north slopes and rocky soils or outcrops that offer some protection from fire.

Cones were maintained separately by trees within populations. For seven populations (Iron Mountain, Little Grayback, Poker Flat, Doolittle Creek, Prescott Cabin, Baldy Mountain, and Rock Creek Butte) staff of the North Zone Tree Improvement Center counted the number of cones. Once the cones dried and opened, seed was extracted by shaking, then hollow seeds were removed by blowing and full seeds weighed. The total number of seeds from each tree was calculated from the relationship between mass and number in samples that were both weighed and counted, providing a measure of full or viable seeds per cone.

Seeds were stored under refrigeration until shipped to the Institute of Forest Genetics, where they were germinated in petri dishes. When radicles emerged, megagametophytes and embryos were dissected from the seeds, separated, and extracted for isozyme electrophoresis. Isozymes from the 1985 samples were analyzed in 1988; the 1989 and 1999 samples were analyzed in 2001.

In spruces, the nutritive tissue of the seed is a haploid megagametophyte that gives rise to, and is of the same genotype as, the egg. Alleles at a locus can be detected by segregation among megagametophytes from a heterozygote. The genotype of the seed parent can be determined by analyzing a number of megagametophytes. When two different alleles at a locus are detected, the seed parent is unequivocally a heterozygote. When only one allele is detected, the tree is classified as a homozygote, although the possibility remains that it is a heterozygote, but by chance the sampled seeds all carried the same allele. The probability of misclassification decreases with increase in sample size. We assayed at least six megagametophytes per tree. For Baldy Mountain and the 1999 sample from Iron Mountain, we analyzed eight megagametophytes. With a sample of six, there is a probability of 0.03125 of misclassifying a heterozygote as a homozygote. That is, the probability that all six megagametophytes in a sample from a heterozygous tree carry the same allele is $2(\frac{1}{2})^6 = 0.03125$. With eight megagametophytes, the probability of misclassifying a heterozygote as a homozygote is only 0.00781. For estimating allele frequencies, the number of parent trees per population, N , varied from 20 to 32 (or $2N = 40$ –64 genomes).

We used techniques of starch gel electrophoresis based on the laboratory manual of Conkle et al. (1982) to assay enzyme systems. In the megagametophytes, we were able to consistently score 26 presumptive loci in 15 enzyme systems in all 10 populations. We interpreted the number of loci and alleles by drawing on the experience gained in our laboratory from studies of allozymes of other conifer species (Conkle, 1981; Ledig et al., 1997, 2000, 2002, 2004). Samples of red pine (*Pinus resinosa* Ait.), which are almost invariably homozygous at all loci, were included as standards on each gel to aid interpretation.

We estimated percentage polymorphic loci, alleles per locus, heterozygosity, and Nei's (1978) unbiased genetic distance with BIOSYS (Swofford and Selander, 1981). For small samples such as ours, BIOSYS calculates unbiased heterozygosity (Nei, 1978). Fixation indices (F) within populations were calculated as the mean deviation of loci from Hardy-Weinberg expectations. We also used BIOSYS to calculate Wright's (1965) F statistics. BIOSYS calculates F_{IS} and F_{IT} , the fixation indices of trees relative to the population and the meta-population, respectively, as weighted averages across alleles. F_{ST} , the proportion of the total genic diversity among populations, is calculated from the relationship: $1 - F_{IT} = (1 - F_{IS})(1 - F_{ST})$. Pairwise values of F_{ST} were calculated using FSTAT2.9.3.2 (Goudet, 2001). All inferences apply to the population of mature, cone-bearing trees.

The degree of genetic isolation among populations was estimated by Nm , the number of migrants per generation. Nm was calculated from Wright (1951) as $Nm = (1 - F_{ST})/4F_{ST}$, and from the number and mean frequency of private alleles, the unique alleles found in only one population (Slatkin, 1985; Barton and Slatkin, 1986).

We used the computer program BOTTLENECK (Cornuet and Luikart, 1996) to determine whether effective population numbers had been restricted in the recent past. We employed the infinite allele model (Kimura and Crow, 1964) because empirically it tends to fit allozyme data better than alternatives (Luikart and Cornuet, 1998). The Wilcoxon sign-rank test was preferred to the sign test because the former has higher power and can be used with as few as four polymorphic loci (Piry et al., 1999). However, we had at least six polymorphic loci available for every population, and in most cases, 10 or more.

The association between geographic and genetic distances was checked using Mantel's (1967) generalized regression procedure. Latitude and longitude of each population sampled was determined with a GPS. Geographic distance between populations was calculated from latitude and longitude using Kindred's (1997) distance calculator.

We investigated phylogeographic relationships among the populations with cluster analysis, using the SEQBOOT, NEIGHBOR, and CONSENSE programs in the PHYLIP package (Felsenstein, 1995). Bootstrapping was performed via random resampling of our data set with the SEQBOOT program to generate 1000 matrices of Nei's (1972) genetic distances. The goal of bootstrapping was to test the consistency with which our data set supported the phenetic relationships among populations. High bootstrap scores (>75%) suggest strong support for a particular cluster, whereas lower levels of support suggest a lower order of differentiation. The unweighted pair group (UPGMA) method was used to produce phylograms using the NEIGHBOR clustering program, and majority-rule consensus trees were generated from bootstrap trees using the program CONSENSE. The phylogenetic trees were viewed and drawn using TREEVIEW (Page, 1996).

The outcrossing rate was estimated for all populations except two, Baldy Mountain and Russian Peak. For mating system analysis, we assayed embryos alongside their megagametophytes. Knowing the contribution of the egg (the haploid genotype of the megagametophyte) to the embryo, the pollen contribution can be deduced by subtraction, so small progeny arrays are adequate to estimate the outcrossing rate with little bias. For three populations (Iron Mountain, Collier Butte, and Little Grayback) that represented a range of site conditions, we assayed seeds from 20 trees each and about 30 megagametophyte-embryo pairs per tree (about 600 progeny per population). In four populations in which the sampled trees were distributed over distances of several kilometers (Poker Flat, Doolittle Creek, Prescott Cabin, and Rock Creek Butte), we assayed seeds from about 50 megagametophyte-embryo pairs per tree from each of four trees in each population (about 200 progeny in each population). Two trees in each of these four populations were relatively isolated and two in each had many near neighbors. In addition to population estimates of outcrossing, we tested whether isolated trees had lower rates of outcrossing than trees with conspecific neighbors. Finally, at Flattop we compared the mating system in 11 trees sampled in 1981, before logging, to nine trees sampled in 1985, after logging. About 60 megagametophyte-embryo pairs were assayed for each of the 20 trees from Flattop (about 1200 progeny).

We used Ritland's (1986, 1989, 1990a, b, 1994) MLTR and MLTF programs to calculate the single- and multiple-locus estimates of the outcrossing

rate, t_s and t_m , at the population level. MLTF was used to estimate t_s and t_m for each polymorphic locus because it uses information on the megagametophyte genotype and the estimate of outcrossing becomes much more accurate (Ritland, 1990a). An exception was 6Pg-2 in Poker Flat; because of the failure of MLTF to return a valid estimate of error, MLTR was used to estimate t_s with 1000 bootstraps. Neither MLTF or MLTR returned a valid estimate of t_s for Pgi-2 in the sample from Prescott Cabin; MLTF failed to return an estimate of error, and in MLTR, estimates converged on whatever initial estimate was used as input or fluctuated between 0.00 and 1.99. Allele frequency and t were always estimated jointly, and the Newton-Raphson method was used for iteration, except in a very few cases where it was necessary to use the expectation maximization method. MLTR was used with 1000 bootstraps to estimate the mean single-locus outcrossing rate (t_s), the correlation of outcrossed paternity (r_p) among progeny within families, and family-level estimates of t_m .

RESULTS

Of the 26 loci consistently scored, 18 were polymorphic in at least one population (Table 1), and eight (*Cat-1*, *Idh-1*, *Mdh-3*, *Mdh-4*, *Mpi*, *Skd-1*, *Skd-2*, *Tpi-2*) were monomorphic in all populations. Many of these monomorphic loci are polymorphic in other spruce species (Ledig et al., 2004).

For most populations, unbiased estimates of expected heterozygosity, H_e , for the 26 loci fell in a narrow range, from 0.113 to 0.161 (Table 2). However, Iron Mountain had an H_e of only 0.059, less than half the mean, and was low in all other measures of diversity. Percentage polymorphic loci, P , ranged from 23.1 to 57.7%, and the number of alleles per locus, A , varied from 1.23 to 1.62. Observed heterozygosity, H_o , was, in general, slightly lower than H_e (Table 2); means for H_e and H_o were 0.129 and 0.121, respectively. All measures of diversity were highly correlated and decreased with increasing latitude (e.g., Fig. 2), with correlation coefficients that ranged from -0.74 for A to -0.94 for H_o .

The mean of the population inbreeding coefficients, 0.052, was low and similar to Wright's F_{IS} , 0.033 (Table 3). Wright's F_{ST} was 0.157, i.e., 15.7% of the total genic diversity was among populations (Table 3). However, much of this variation resulted from inclusion of the northern isolate, Iron Mountain. Mean pairwise F_{ST} between Iron Mountain and the other nine populations was 0.356 ± 0.062 . Pairwise F_{ST} values among the other nine populations averaged only 0.103 ± 0.071 . The number of migrants per generation, Nm , estimated from Wright's F_{ST} was only 1.34. Nm estimated from the mean frequency of private alleles for a sample size of 25 and corrected for the actual mean sample of 21.6, was twice as large, 2.70.

Nei's unbiased genetic distance (D) reflected the differences in Nm (Table 4). Mean D between Iron Mountain and the other nine populations was 0.055 ± 0.015 , more than twice as great as the mean among those populations, 0.019 ± 0.014 . Pairwise D values were not related to distance between populations. The Mantel matrix correlation between D and geographic distance was a nonsignificant 0.34 (probability = 0.101, based on 10000 permutations). The Mantel correlation between F_{ST} and geographic distance was 0.37, which though statistically significant (probability = 0.050) was scarcely different than the correlation of D and geographic distance. D and F_{ST} are both measures of differentiation and their correlations with geographic distance should be consistent. The correlations virtually disappeared (i.e., r was approximately 0.0) if the two most isolated and divergent populations, Iron Mountain and Collier Butte, were omitted from the analyses.

UPGMA revealed some structure among Brewer spruce

TABLE 1. Allele frequencies for 18 polymorphic loci in 10 populations of Brewer spruce.

Locus Allele		Population ^a (Sample size ^b)									
		IM (64)	Ft (40)	CB (42)	LG (42)	PF (40)	DC (40)	PC (44)	BM (40)	RCB (42)	RP (42)
<i>Fest</i>	1	1.000	0.975	1.000	1.000	1.000	0.975	1.000	1.000	1.000	1.000
	2	0.000	0.025	0.000	0.000	0.000	0.025	0.000	0.000	0.000	0.000
<i>Gdh</i>	1	1.000	0.947	1.000	0.857	0.900	0.850	0.841	0.975	0.929	0.976
	2	0.000	0.053	0.000	0.143	0.100	0.150	0.159	0.025	0.071	0.024
<i>Got-1</i>	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.976
	2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.024
<i>Got-3</i>	1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.929	1.000
	2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.071	0.000
<i>Idh-2</i>	1	1.000	1.000	1.000	0.976	0.900	1.000	1.000	1.000	0.976	0.900
	2	0.000	0.000	0.000	0.024	0.100	0.000	0.000	0.000	0.024	0.100
<i>Lap-1</i>	1	1.000	1.000	0.976	1.000	0.842	1.000	0.818	1.000	0.952	0.952
	2	0.000	0.000	0.024	0.000	0.158	0.000	0.114	0.000	0.048	0.048
<i>Mdh-1</i>	1	1.000	0.975	1.000	1.000	0.975	1.000	0.952	1.000	0.976	0.975
	2	0.000	0.025	0.000	0.000	0.025	0.000	0.048	0.000	0.024	0.025
<i>Mdh-2</i>	1	0.906	0.100	0.833	0.167	0.225	0.475	0.636	0.400	0.357	0.200
	2	0.094	0.900	0.167	0.833	0.775	0.525	0.364	0.600	0.643	0.800
<i>Mdh-5</i>	1	0.984	0.750	0.450	0.762	0.575	0.850	0.452	0.575	0.429	0.550
	2	0.016	0.250	0.550	0.238	0.425	0.150	0.548	0.425	0.571	0.450
<i>Mdh-6</i>	1	1.000	0.975	1.000	1.000	0.875	1.000	0.952	1.000	0.952	0.975
	2	0.000	0.025	0.000	0.000	0.125	0.000	0.048	0.000	0.048	0.025
<i>Mnr-1</i>	1	1.000	0.975	0.975	1.000	1.000	0.975	1.000	1.000	1.000	1.000
	2	0.000	0.025	0.025	0.000	0.000	0.025	0.000	0.000	0.000	0.000
<i>6Pg-2</i>	1	0.578	0.475	0.714	0.452	0.325	0.575	0.667	0.750	0.476	0.525
	2	0.422	0.525	0.238	0.548	0.675	0.425	0.333	0.250	0.524	0.475
	3	0.000	0.000	0.048	0.000	0.000	0.000	0.000	0.000	0.000	0.000
<i>Pgi-1</i>	1	0.063	0.025	0.000	0.024	0.000	0.000	0.023	0.225	0.000	0.024
	2	0.938	0.975	1.000	0.976	1.000	1.000	0.977	0.775	1.000	0.976
<i>Pgi-2</i>	1	0.125	0.450	0.905	0.381	0.350	0.625	0.636	0.500	0.381	0.548
	2	0.875	0.550	0.095	0.619	0.650	0.375	0.364	0.500	0.619	0.452
<i>Pgm</i>	1	0.000	0.475	0.548	0.190	0.325	0.200	0.333	0.150	0.571	0.500
	2	1.000	0.525	0.452	0.810	0.675	0.800	0.667	0.850	0.429	0.500
<i>Tpi-1</i>	1	1.000	1.000	0.550	0.976	0.975	0.950	0.810	0.950	0.905	0.850
	2	0.000	0.000	0.450	0.024	0.025	0.050	0.190	0.050	0.095	0.150
<i>Ugp-1</i>	1	1.000	0.250	0.725	0.548	0.775	0.475	0.190	0.375	0.357	0.625
	2	0.000	0.750	0.275	0.452	0.200	0.500	0.738	0.625	0.619	0.275
<i>Ugp-2</i>	3	0.000	0.000	0.000	0.000	0.025	0.025	0.071	0.000	0.024	0.100
	1	0.469	0.125	0.000	0.310	0.050	0.175	0.048	0.150	0.095	0.400
	2	0.531	0.850	1.000	0.690	0.950	0.825	0.952	0.800	0.905	0.600
	3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.050	0.000	0.000
	4	0.000	0.025	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

^a IM = Iron Mountain; Ft = Flattop; CB = Collier Butte; LG = Little Grayback; PF = Poker Flat; DC = Doolittle Creek; PC = Prescott Cabin; BM = Baldy Mountain; RCB = Rock Creek Butte; RP = Russian Peak.

^b 2N, the number of genomes, or twice the number of trees.

TABLE 2. Genic diversity and fixation index in 10 Brewer spruce populations.

Population	N	H _e	H _o	P	P ₉₅	A	F
Iron Mountain	31.9	0.059 (0.028)	0.063 (0.030)	23.1	19.2	1.23 (0.08)	-0.068
Flattop	19.9	0.119 (0.036)	0.100 (0.030)	50.0	30.8	1.54 (0.11)	0.160
Collier Butte	20.6	0.113 (0.038)	0.101 (0.035)	34.6	26.9	1.38 (0.11)	0.106
Little Grayback	21.0	0.127 (0.037)	0.126 (0.037)	42.3	30.8	1.42 (0.10)	0.008
Poker Flat	20.0	0.141 (0.036)	0.127 (0.034)	50.0	42.3	1.54 (0.11)	0.099
Doolittle Creek	20.0	0.130 (0.038)	0.125 (0.037)	42.3	34.6	1.46 (0.11)	0.038
Prescott Cabin	21.4	0.155 (0.039)	0.147 (0.039)	50.0	34.6	1.58 (0.13)	0.052
Baldy Mountain	20.0	0.134 (0.039)	0.135 (0.040)	38.5	34.6	1.42 (0.11)	-0.007
Rock Creek Butte	21.0	0.149 (0.039)	0.137 (0.038)	53.8	38.5	1.58 (0.13)	0.081
Russian Peak	20.3	0.161 (0.042)	0.153 (0.041)	57.7	34.6	1.62 (0.11)	0.050
Mean		0.129	0.121	44.2	32.7	1.48	0.052

Note: N = mean no. of trees sampled per locus at 26 loci; H_e = expected heterozygosity (unbiased estimate); H_o = observed heterozygosity; P = percentage polymorphic loci; P₉₅ = percentage polymorphic loci at 95% criterion; A = number of alleles per locus; F = fixation index; standard errors in parentheses.

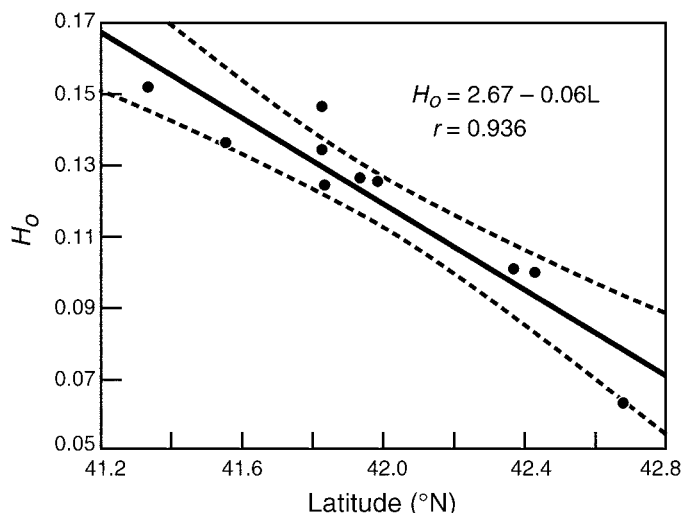


Fig. 2. Decrease in observed heterozygosity with latitude in Brewer spruce. Dashed lines are 95% confidence intervals around the regression.

populations, but the relationships were not strongly supported (Fig. 3). Two populations with high genic diversity, Russian Peak and Poker Flat, were near the root of the phylogram, which suggests that they may be closest to ancestral Brewer spruce. The two northwestern isolates, Iron Mountain and Collier Butte, formed a cluster at the top of the phylogram. Although weakly supported by the bootstrap analysis, the cluster of Baldy Mountain and Doolittle Creek was reasonable because these two populations were very close, Baldy Mountain being higher in elevation than Doolittle Creek but on the same slope. This cluster was then joined by Prescott Cabin, which is near Baldy Mountain and Doolittle Creek. The positions of Rock Creek Butte, Flattop, and Little Grayback were not so easily rationalized.

In general, the Wilcoxon sign-rank test indicated no greater heterozygosity than that expected for populations at mutation-drift equilibrium (Table 5), suggesting that these populations have not been bottlenecked recently. The exception was Baldy

Mountain, where the probability of 0.042 was significant. However, statistical significance of one test among 11 must be regarded with caution.

The multilocus, population outcrossing rates for the three populations in which we sampled 20 progeny arrays (Iron Mountain, Collier Butte, and Little Grayback) ranged from 0.926 to 0.959 (Table 6). Likewise, outcrossing rates were fairly high for the four populations in which only four progeny arrays were sampled (Poker Flat, Doolittle Creek, Prescott Cabin, Rock Creek Butte), ranging from 0.750 to 1.000. For three of these populations, $t_m \geq 0.87$. Although a value of 0.750 for Rock Creek Butte clearly indicates a mixed mating system, the 95% confidence interval includes 0.830, and in each of these four populations, half of the four progenies sampled represented isolated trees, which might have biased t_m downward. The mean single-locus outcrossing rates were often as great as or greater than the multilocus rates and never significantly lower than the multilocus rate, suggesting that little inbreeding, in addition to that contributed by selfing, occurred by crossing among relatives. The correlation of outcrossed paternity, r_p , among progeny within a family ranged from low to moderately high, which suggests that some populations sampled a diverse pollen pool, while others were pollinated by a more limited number of fathers (Table 6).

The post-logging outcrossing rate at Flattop was significantly lower than the pre-logging rate; $t_m = 0.756 \pm 0.028$ post-logging vs. 0.961 ± 0.018 pre-logging (Table 6). As a result, the inbreeding coefficient of the 660 embryos sampled prior to logging was 0.000, vs. 0.161 for the 540 embryos sampled after logging.

No difference in t_m was observed between isolated trees vs. those with near neighbors in the Poker Flat, Doolittle Creek, Prescott Cabin, or Rock Creek Butte samples (Table 7). In fact, outcrossing estimates were higher for isolated trees than for trees with immediate neighbors at Poker Flat and Rock Creek Butte, although the difference was not significant.

Among the seven populations for which seed and cone counts were available, differences in seeds per cone were very highly significant according to an analysis of variance (Fig. 4). Poker Flat, Prescott Cabin, and Rock Creek Butte had much lower seed counts per cone than the denser populations, Iron Mountain, Little Grayback, and Baldy Mountain. Doolittle Creek was intermediate.

TABLE 3. Estimates of Wright's (1965) F statistics for 18 polymorphic loci in Brewer spruce.

Locus	F_{IS}	F_{IT}	F_{ST}
<i>Fest</i>	-0.026	-0.005	0.020
<i>Gdh</i>	-0.066	-0.011	0.052
<i>Got-1</i>	-0.024	-0.002	0.021
<i>Got-3</i>	-0.077	-0.007	0.065
<i>Idh-2</i>	-0.093	-0.025	0.062
<i>Lap-1</i>	0.224	0.289	0.084
<i>Mdh-1</i>	-0.033	-0.015	0.018
<i>Mdh-2</i>	-0.044	0.260	0.291
<i>Mdh-5</i>	0.113	0.237	0.139
<i>Mdh-6</i>	0.306	0.343	0.054
<i>Mnr-1</i>	-0.026	-0.008	0.018
<i>6Pg-2</i>	-0.094	-0.022	0.066
<i>Pgi-1</i>	-0.021	0.096	0.114
<i>Pgi-2</i>	0.105	0.246	0.157
<i>Pgm</i>	0.024	0.171	0.151
<i>Tpi-1</i>	0.285	0.416	0.183
<i>Ugp-1</i>	-0.008	0.215	0.221
<i>Ugp-2</i>	0.051	0.189	0.146
Mean	0.033	0.186	0.157

DISCUSSION

Genic diversity—The estimates of genic diversity in Brewer spruce were much higher than average for long-lived, woody endemics and close to average for outcrossing endemic plant species in general. Percentage polymorphic loci (P) in Brewer spruce was about 44%, which is less than the mean for outcrossing endemics (54.4%) but higher than that for woody endemics (26.3%) reviewed by Hamrick and Godt (1996b) and Hamrick et al. (1992). Mean expected heterozygosity (H_e) in Brewer spruce averaged 0.129 compared to 0.056 for 20 long-lived woody endemics (Hamrick et al., 1992) and 0.142 for 57 outcrossing endemic plant species (Hamrick and Godt, 1996b). H_e in Brewer spruce was about 15% lower than values for gymnosperms in general (0.151) or outcrossing, wind-pollinated trees (0.154) reviewed by Hamrick et al. (1992). However, if the genically depauperate Iron Mountain is omitted, mean H_e for the other nine populations of Brewer spruce is 0.137, and at Russian Peak, H_e is 0.161.

TABLE 4. Half-matrices of Nei's (1978) unbiased genetic distances (D , above diagonal) calculated from frequencies of 26 loci scored in megagametophytes and geographic distances (in km, below diagonal) between pairs of Brewer spruce populations.

Population ^a	IM	Ft	CB	LG	PF	DC	PC	BM	RCB	RP
IM		0.073	0.073	0.039	0.046	0.035	0.068	0.046	0.066	0.052
Ft	40		0.058	0.006	0.016	0.011	0.021	0.013	0.006	0.058
CB	35	29		0.058	0.048	0.031	0.020	0.036	0.034	0.035
LG	92	54	66		0.007	0.005	0.027	0.010	0.014	0.007
PF	97	59	69	7		0.016	0.030	0.021	0.011	0.009
DC	110	72	81	18	13		0.012	0.006	0.016	0.014
PC	102	69	70	25	19	20		0.009	0.009	0.024
BM	111	74	82	20	14	3	19		0.012	0.015
RCB	132	99	100	50	44	35	30	32		0.008
RP	181	142	153	88	84	72	85	71	65	

^a IM = Iron Mountain; Ft = Flattop; CB = Collier Butte; LG = Little Grayback; PF = Poker Flat; DC = Doolittle Creek; PC = Prescott Cabin; BM = Baldy Mountain; RCB = Rock Creek Butte; RP = Russian Peak.

Compared to other endemic spruces of western North America, Brewer spruce is at least as diverse; for 10 populations of Chihuahua spruce (*Picea chihuahuana* Martínez), a species endemic to the Sierra Madre Occidental, H_e averaged only 0.093 (Ledig et al., 1997); for two populations of Martínez spruce (*Picea martinezii* T.F. Patterson), a very rare endemic of the Sierra Madre Oriental, H_e was 0.101 and 0.121 (Ledig et al., 2000); and for the only three known populations of Mexican spruce (*Picea mexicana* Martínez; Ledig et al., 2002), H_e ranged from 0.117 to 0.130.

Russian Peak is the only area where Brewer spruce is sympatric with any congener. An isolated population of Engelmann spruce is found below and mixed with Brewer spruce at its lowest elevation on Russian Peak. However, hybridization does not seem to be the reason for the high heterozygosity observed in Brewer spruce. No natural hybrids between Brewer and Engelmann spruces have been reported and controlled crosses have failed completely (Gordon, 1986). The two species are distinct in their isozyme profiles (Ledig et al., 2004).

Perhaps the most notable point about genic diversity in Brewer spruce was the decrease in heterozygosity with increase in latitude. Similar trends in other species, plant and animal, have been interpreted as the trace of postglacial dispersion northward (Gooch and Glazier, 1986; Cwynar and MacDonald, 1987; Moran et al., 1989; Hamrick and Godt, 1996a; Ledig, 2000). In Coulter pine (*Pinus coulteri* D. Don),

the trace is unequivocal, marked by the progressive loss of alleles along a route from the San Jacinto Mountains of southern California to the Diablo Range in the north (Ledig, 2000). Although a few alleles in the southern populations of Brewer spruce disappear northward, the pattern is irregular and suggests random genetic drift in small populations rather than founder effects. The northern isolates, Iron Mountain and Collier Butte, are homozygous at several loci (Table 1), but this may be attributed to their small size and isolation equally as well as to founder effects arising from dispersal northward during the Holocene or previous interglacials. A general dispersal northward conflicts with the interpretation of Brewer spruce as an Arcto-Tertiary relict that has persisted in the Klamath Region, perhaps since the Miocene (Whittaker, 1961; Wolfe, 1969; Sawyer and Thornburgh, 1977).

A Wahlund effect might be expected in several of the "populations" sampled because the trees were distributed over distances exceeding a kilometer in some cases, and even up to 5 km. This does not seem to be the case; inbreeding coefficients were generally low. One of the largest estimates of F was for Collier Butte, a population in which samples were confined within a relatively small area and unlikely to show a Wahlund effect. Where a strong Wahlund effect might have been expected (i.e., for Poker Flat, Doolittle Creek, Prescott Cabin, Baldy Mountain, and Rock Creek Butte), estimates of F ranged only from -0.007 to 0.099 .

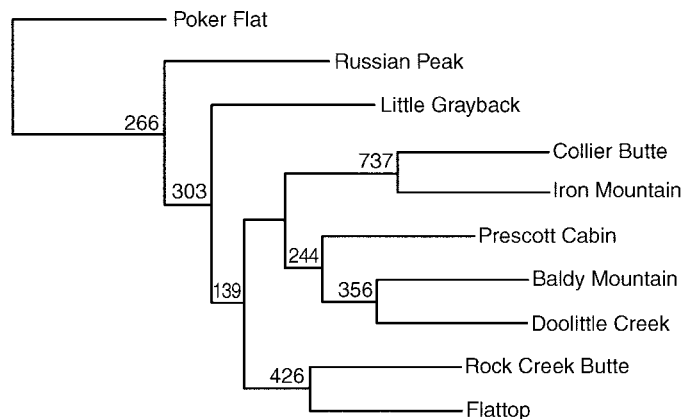


Fig. 3. Consensus (UPGMA) tree constructed from 1000 trees based on Nei's genetic distances among 10 populations of Brewer spruce; no. near nodes represents the number of times the node occurred in the 1000 bootstrap-generated data sets.

TABLE 5. Wilcoxon sign-rank test for recent bottlenecks in 10 Brewer spruce populations under the infinite allele model of mutation-drift equilibrium.

Population	No. of loci	N^a	Prob. ^b
Iron Mountain	6	64.0	0.422
Flattop 1981	10	22.0	0.116
Flattop 1985	10	18.0	0.615
Collier Butte	9	41.1	0.082
Little Grayback	11	42.0	0.103
Poker Flat	13	39.8	0.249
Doolittle Creek	11	40.0	0.139
Prescott Cabin	13	42.8	0.137
Baldy Mountain	10	40.0	0.042
Rock Creek Butte	14	42.0	0.232
Russian Peak	15	40.7	0.445

^a Number of genomes sampled.

^b Wilcoxon sign-rank test one-tailed probability of heterozygote excess under the infinite allele model of mutation-drift equilibrium (Kimura and Crow, 1964).

TABLE 6. Single locus (t_s) and multilocus (t_m) population outcrossing rates and the correlation of outcrossed paternity (r_p) in eight populations of Brewer spruce (standard errors in parentheses).

Locus	Flattop		Collier Butte (20; 594.5)	Little Grayback (20; 552.0)	Poker Flat (4; 186.5)	Doolittle Creek (4; 198.9)	Prescott Cabin (4; 178.8)	Rock Creek Butte (4; 199.4)
	1981 (11; 657.6)	1985 (9; 539.0)						
<i>Gdh</i>	—	—	—	0.924 (0.091)	1.028 (0.067)	0.868 (0.100)	0.823 (0.131)	—
<i>Got-1</i>	—	—	—	—	—	—	0.805 (1.592)	—
<i>Lap-2</i>	—	—	—	—	0.430 (0.096)	0.558 (0.157)	—	—
<i>Mdh-2</i>	0.852 (0.068)	0.915 (0.059)	0.743 (0.063)	0.904 (0.060)	0.819 (0.076)	1.376 (0.093)	0.924 (0.161)	0.522 (0.117)
<i>Mdh-3</i>	—	—	—	0.851 (0.108)	—	—	—	—
<i>Mdh-5</i>	—	—	1.109 (0.122)	—	0.931 (0.205)	—	0.481 (0.202)	—
<i>6Pg-1</i>	—	—	—	—	1.106 (0.085)	0.339 (0.110)	0.902 (1.663)	0.730 (0.081)
<i>6Pg-2</i>	0.929 (0.061)	0.966 (0.058)	0.997 (0.040)	0.919 (0.058)	1.816 (0.068)	0.462 (0.132)	0.829 (0.069)	0.705 (0.067)
<i>Pgi-2</i>	1.019 (0.048)	0.922 (0.045)	0.942 (0.080)	0.963 (0.059)	1.999 (0.017)	1.011 (0.082)	— ^b	0.481 (0.153)
<i>Pgm</i>	—	—	0.953 (0.058)	—	—	—	0.472 (0.084)	—
<i>Tpi-1</i>	—	—	—	—	—	—	0.867 (2.128)	—
<i>Ugp-1</i>	—	1.114 (0.047)	0.816 (0.061)	0.766 (0.053)	0.813 (0.140)	0.729 (0.090)	1.227 (0.121)	0.671 (0.078)
<i>Ugp-4</i>	0.911 (0.057)	—	—	0.831 (0.057)	—	—	0.854 (1.515)	—
Mean t_s	0.953 (0.046)	0.761 (0.090)	0.987 (0.047)	0.942 (0.038)	0.929 (0.082)	0.929 (0.082)	0.825 (0.101)	0.721 (0.080)
t_m	0.959 (0.038)	0.756 (0.028)	0.941 (0.025)	0.926 (0.020)	1.000 (0.033)	0.965 (0.028)	0.874 (0.035)	0.750 (0.040)
r_p	0.137 (0.064)	0.070 (0.049)	0.538 (0.142)	0.474 (0.114)	0.574 (0.286)	0.175 (0.162)	0.482 (0.178)	0.077 (0.225)

^a No. of progeny arrays; mean no. of embryos observed per locus.
^b Estimate unreliable.

TABLE 7. Multilocus, family outcrossing rates (and standard errors based on 1000 bootstraps) estimated from MLTR (except as noted) for two isolated and two clustered trees in each of four populations of Brewer spruce.

Population	Isolated	Clustered
Poker Flat	0.99 (0.02)	0.98 (0.03)
	1.13 (0.26)	1.19 (0.26) ^a
Doolittle Creek	0.98 (0.23)	0.96 (0.23)
	1.20 (0.30)	1.01 (0.34)
Prescott Cabin	0.53 (0.08)	1.00 (0.21)
	0.98 (0.13)	0.91 (0.07)
Rock Creek Butte	1.71 (0.33)	0.92 (0.46)
	1.06 (0.41)	0.66 (0.07)
Mean	1.06	0.95

^a Estimated with MLTF.

Genetic structure—Brewer spruce may be characterized as moderately structured. Even excluding the outlier, Iron Mountain, F_{ST} was 0.103 among the other nine populations, which is high for conifers. Only two of 18 studies of north temperate and boreal spruces reported estimates of F_{ST} greater than 0.10 (reviewed in Ledig et al., 1997), and none as high as the estimate of 0.157 when Iron Mountain was included. Only in Chihuahua spruce, with its highly fragmented distribution, was F_{ST} larger, 0.248 (Ledig et al., 1997).

Gene exchange among Brewer spruce populations was low relative to many conifers; the estimate of Nm based on F_{ST} was 1.34. Because estimates of Nm do not reach equilibrium for perhaps 100–1000 generations after gene flow ceases (Slatkin and Barton, 1989), an Nm of 1.34 is likely to reflect past contact, not the present rate of migration. By comparison, Nm in red spruce (*Picea rubens* Sarg.) was 20.1 in Canada's Maritime Provinces and 3.0 in Ontario (Rajora et al., 2000). A high F_{ST} and low Nm in Brewer spruce suggest the possibility of differentiation by random genetic drift. This interpretation was further supported by the lack of relationship between genetic distance and geographic distance, which would have been expected if gene flow was extensive because near neighbors are more likely to exchange genes than distant popula-

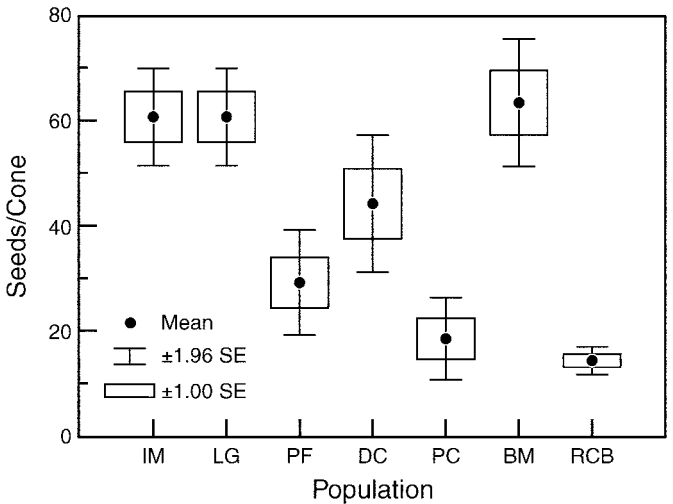


Fig 4. Means, standard errors, and 95% confidence intervals for number of seeds per cone in seven Brewer spruce populations (IM = Iron Mountain; LG = Little Grayback; PF = Poker Flat; DC = Doolittle Creek; PC = Prescott Cabin; BM = Baldy Mountain; RCB = Rock Creek Butte).

tions. Although some near neighbors clustered on an UPGMA tree calculated from Nei's genetic distance (Fig. 3), the tree revealed little phylogeographic pattern that would suggest isolation by distance through a stepping stone model. The relationship between F_{ST} or D and the geographic distance between pairs of populations suggests little gene flow between near neighbors. Despite a genetic structure influenced by drift, a test for recent bottlenecks found no greater heterozygosity than that expected for populations at mutation-drift equilibrium, except for Baldy Mountain.

Mating systems—The mixed mating model for the estimation of outcrossing rate assumes loci are in linkage equilibrium. Our samples were too small to estimate disequilibrium or even the recombination rate between loci. However, single-locus estimates are not affected by linkage disequilibrium, and the means of the single-locus estimates, t_s , of outcrossing were similar to estimates of t_m . Therefore, linkage probably had little effect on the estimate of t_m reported here.

Differences between single-locus and multilocus estimates of outcrossing can be used to infer crossing between relatives that is in addition to inbreeding resulting from self-pollination. Crosses between relatives may occur as a result of limited dispersion of progeny around seed trees followed by pollination between neighbors. In our samples, t_s was slightly greater than t_m in many cases and never significantly less, so there is no evidence of crossing among relatives.

Neither is there evidence of much selfing from the mating system analyses. Given the spatial structure of Brewer spruce populations, the rate of outcrossing seems, in general, quite high. Brewer spruce typically occurs in mixed forest under larger true firs and Douglas-fir, which would seem to impose a barrier to exchange of pollen. However, outcrossing was as high in mixed conifer stands (e.g., $t_m = 0.926 \pm 0.020$ at Little Grayback and 0.961 ± 0.018 at Flattop before logging) as it was in almost pure stands dominated by Brewer spruce (e.g., $t_m = 0.959 \pm 0.028$ at Iron Mountain).

All populations of Brewer spruce, even in samples represented in part by isolated trees (Poker Flat, Doolittle Creek, Prescott Cabin, and Rock Creek Butte), had higher rates of outcrossing than other endemic spruces. Two small populations of Chihuahua spruce had outcrossing rates of 0 and 15% (Ledig et al., 1997); rates in Mexican spruce ranged from 59 to 81% (Ledig et al., 2002); and rates in Martínez spruce ranged from 40 to 69% (Ledig et al., 2000). Too little information exists on any of these species to speculate on the reasons for the comparatively high level of outcrossing in Brewer spruce.

Outcrossing rates in Brewer spruce were typical of wide-ranging species that grow in large, uninterrupted populations, where estimates generally range from 84 to 98% (King et al., 1984; Cheliak et al., 1985; Shea, 1987; Kuittinen and Savolainen, 1992; Chaisurisri et al., 1994). Likewise, the rare Serbian spruce [*Picea omorika* (Panc.) Purk.] whose populations occupy, in total, only 60 ha in its native range and which is almost entirely self-fertile has an outcrossing rate of 84% (Kuittinen and Savolainen, 1992). Exceptions to these high rates of outcrossing are Norway spruce [*Picea abies* (L.) Karst.] at the northern limits of its range in Finland (Muona et al., 1990) and white spruce [*Picea glauca* (Moench) Voss] in Newfoundland (Innes and Ringius, 1990), in which relatively low levels of outcrossing were observed (74 and 73%, respectively). In red spruce, which has a regional distribution

in Canada and the United States, Rajora et al. (2000) observed outcrossing rates of only 65% in Ontario and 54% in the Maritime provinces. However, these estimates were based on ungerminated seeds, and Rajora et al. (2000) pointed out that allozyme-based estimates from germinable seeds measure only "tolerable" inbreeding events, which is the case in our study of Brewer spruce.

Even the Brewer spruce trees chosen for analysis because of their isolated condition seemed to have fairly high rates of outcrossing, with an estimated mean t_m of 1.06 (100%) for eight isolated trees (Table 7). This suggests either a copious pollen cloud or some mechanism(s) for the prevention of inbreeding, such as self-infertility or incompatibility. Incompatibility, however, is unknown in the Pinaceae.

The equilibrium fixation index, F_e , can be calculated from the outcrossing rate, t , as (Allard et al., 1968): $F_e = (1 - t)/(1 + t)$. The relatively low estimates of F were consistent with the observed high rates of outcrossing, especially for the four populations in which estimates of t_m were based on samples of 20 trees.

r_p is the probability that a randomly chosen pair of embryos from the same seed tree were full-sibs. Values around 0.10 (e.g., Iron Mountain, Flattop, and Rock Creek Butte in Table 6) suggest at least 10 pollen parents in the progeny array if all were equally represented, a fairly diverse pollen pool. Higher values, around 0.50 (e.g., Collier Butte, Little Grayback, Poker Flat, and Prescott Cabin) suggest fewer pollen parents per progeny array, only about two if each was equally represented. A high r_p in these populations suggests that most trees sampled a limited pollen pool, composed, perhaps, of a few neighbors. However, standard errors for r_p were high, so any conclusions are weak.

Given the unexpectedly high outcrossing rates of isolated trees, the low post-logging outcrossing rate at Flattop was surprising. No heterozygote deficiency was observed among progeny in the pre-logging sample, but the inbreeding coefficient for progeny sampled after logging was 0.161. A lower outcrossing rate might be anticipated in closed stands than in stands opened by logging. The position of Brewer spruces as subdominants scattered below Douglas-firs and other species, as at Little Grayback, might be expected to reduce dispersal of Brewer spruce pollen because air movement beneath canopies is less than that above the canopy. The stems and crowns of Brewer spruce's taller associates in unlogged stands might constitute a barrier to pollen exchange and, therefore, to outcrossing. This does not seem to be the case. Instead, outcrossing was reduced when the stand at Flattop was opened, even though the frequency of Brewer spruce increased relative to its associated species. A reduction in density of Brewer spruce after logging does not seem a likely explanation for the increase in selfing because the relatively isolated trees in other populations had higher rates of outcrossing than did the post-logging Brewer spruces at Flattop.

Dwarf mistletoe might be an explanation. Brewer spruce can survive overstory removal, but evaporative demand increases and results in early stomatal closure and vigor reduction (Waring et al., 1975). This may stress the trees and favor mistletoe infestation. Cone production in heavily infested trees was noticeably reduced or eliminated entirely. Mistletoe may also reduce the number of pollen strobili and, therefore, the pollen cloud, but this has not been quantified. In any case, the increase in selfing suggests that overstory removal by logging

may be detrimental to regeneration of Brewer spruce, and create a problem in maintaining the species.

Seed yields—Seeds per cone should reflect the rate of selfing in the Pinaceae. In most conifers that have been experimentally pollinated, seed yields were considerably reduced by self-pollination relative to cross-pollination (Kraus and Squilace, 1964; Franklin, 1970; Coles and Fowler, 1976; Ledig, 1986). Trees at Poker Flat, Prescott Cabin, and Rock Creek Butte were sparsely distributed over substantial distances, and the numbers of seeds per cone were much lower than at Iron Mountain, Little Grayback, or Baldy Mountain (Fig. 4). Iron Mountain and Little Grayback had comparatively dense populations of Brewer spruce, and at Baldy Mountain, 17 of 20 trees were within a relatively dense group. The difference in numbers of seeds per cone might suggest more self-pollination within Poker Flat, Prescott Cabin, and Rock Creek Butte than within the three denser populations. The situation at Doolittle Creek, where cone-bearing trees were spread over long distances, similar to the situation at Poker Flat, Prescott Cabin, and Rock Creek Butte, is less obvious. Numbers of seeds per cone at Doolittle Creek were intermediate between those in the relatively dense populations and those populations in which trees were sparsely distributed.

Numbers of seeds per cone were unrelated to a tree's outcrossing rate, either overall ($r = -0.04$, $n = 159$) or within Iron Mountain or Little Grayback, populations for which we had outcrossing estimates for 20 and 19 trees, respectively. Estimating outcrossing from viable seeds (i.e., those that germinate) measures the effective rate of inbreeding within the surviving progeny, but not the actual rate of self-pollination in the ovules. It appears that almost no inbreds in Brewer spruce survive embryogeny, which suggests a high genetic load. In the Pinaceae, the genetic load of recessive lethal equivalents is usually high and selfing results in fully developed seed coats in which the embryo has aborted (Sorensen, 1969; Bramlett and Popham, 1971; Koski, 1971; Franklin, 1972; Bishir and Pepper, 1977; Park and Fowler, 1984; Ledig, 1986). High effective outcrossing rates but low seed yields are consistent with the suggestions from paleontology that Brewer spruce is an ancient species that might be expected to have accumulated a high load of embryonic lethal equivalents over millions of years. Even though only a few inbred progeny survive to the seedling stage and inbreeding coefficients are low, self-pollination is detrimental because it reduces reproductive output by depressing seed yields.

Variance among trees in seed production may also contribute to random genetic drift because it reduces effective population size. For example, only six full seeds were obtained from 52 cones of one relatively isolated tree in the Prescott Cabin population (0.12 seeds per cone), while another tree produced 1938 seeds from 25 cones (77.5 per cone). High variance in number of offspring reduces effective population size (N_e) as $N_e = 4N/(2 + \sigma_i^2)$, where σ_i^2 is the variance in family size. If variance among trees in the number of seeds per cone reflects variance in number of offspring, effective population sizes could be about one-hundredth of the observed population sizes. The ratio of N_e to N in Brewer spruce populations estimated from the variance in number of seeds per cone was about 0.005 to 0.015, except for Rock Creek Butte, where the variance in seeds per cone was small (see Fig. 4) and N_e/N was 0.1.

Conservation of Brewer spruce—Relatively high genetic diversity and rates of outcrossing are positive factors for the conservation of Brewer spruce. However, some of the most isolated populations of Brewer spruce have limited diversity, most notably Iron Mountain, and low diversity may be a problem under projected climate change scenarios or if other environmental changes threaten. In general, diversity increases southward, suggesting that populations in California are most genetically robust. Although genetic structure, as indicated by F_{ST} , is high, it is not as pronounced as in the endemic spruces of México, which suggests that most of the genetic diversity in Brewer spruce could be captured in relatively few populations. In fact, most of the observed structure resulted from inclusion of the northern outliers on Iron Mountain and Collier Butte, and gene flow seems to have provided at least a minimum of connection among the other populations. Outcrossing rates were surprisingly high, especially considering the low rates found in other spruces with fragmented distributions. Outcrossing was high even in isolated trees, which is puzzling and suggests either strong mechanisms for the avoidance of inbreeding or effective local pollen dispersal exceeding that encountered in other spruces of southwestern North America (Jackson, 1994; Jackson and Smith, 1994). Pollen flight and reproductive biology of Brewer spruce might be a fruitful area of research.

On one hand, the high rates of outcrossing and low estimates of inbreeding are welcome news for the conservation of Brewer spruce. However, a mating system analysis measures only effective inbreeding. The differences in seed yields among populations suggests extensive self-pollination in populations of sparsely distributed trees, and low seed yields could be a barrier to natural regeneration of Brewer spruce and reduce possibilities for dispersal to new habitat.

The most troubling result for conservation of this living fossil was the impact of logging on outcrossing rate. The increase in selfing in a heavily logged stand may be related to mistletoe infection. In any case, observation indicated that mistletoe reduced or eliminated cone production, which is bound to interfere with regeneration of the species. Further research should be conducted to verify whether opening stands by logging is a threat to Brewer spruce and to what degree low seed yields resulting from selfing is a problem for natural regeneration.

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