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Research Article

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Table Mountain Pine (*Pinus pungens*): Genetic Diversity and Conservation of an Imperiled Conifer

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Abstract

Table Mountain pine (*Pinus pungens* Lamb.) is an imperiled tree species endemic to the southern and central Appalachian Mountains. Generally reliant on fire for regeneration, its fragmented but widespread distribution has declined in recent decades. We quantified the genetic diversity of 26 populations across the range of the species using data from seven highly polymorphic simple sequence repeat (SSR) loci. The species was relatively inbred whereas differentiation among populations was relatively low. Differentiation was significantly but weakly associated with geographic distance among populations. We detected minor genetic differences between northern and southern seed collection zones established based on climate similarity. We conducted a series of simulations using SSR data from 498 seedlings, grown from seed collected from five natural stands of Table Mountain pine, to assess the genetic consequences of different strategies for deploying collected seed in *ex situ* conservation plantings. Results indicated that reducing the number of families in a planting would not substantially affect the conservation of common alleles but would affect the representation of rare alleles and overall allelic richness. These findings add to our limited knowledge of genetic variation across the distribution of this rare conifer and offer some guidance for its effective genetic conservation.

Study Implications: Most of the results of this study point to an overall lack of genetic structure and geographic differences in genetic variation within Table Mountain pine, most likely the consequence of prolific interpopulation gene flow. The weak genetic differences between northern and southern seed zones indicate that they are not particularly useful for guiding future gene conservation efforts. The results of seedling sampling simulations demonstrated that it may be possible to meet the goals of conservation plantings by including fewer families per population and/or including families without regard to population.

Keywords: Gene conservation, inbreeding, microsatellite, population isolation, rare species, sampling

Table Mountain pine (*Pinus pungens* Lamb.) is a conifer species endemic to the Appalachian Mountains of the Eastern United States, generally inhabiting elevations of 305–1,220 m on well-drained and rocky southern to western-facing ridge and mountain sites (Zobel 1969; Della-Bianca 1990; Williams 1998). Closely related to two more widespread pine species of the southeastern US, pitch pine (*P. rigida* Mill.) and pond pine (*P. serotina* Michx.) (Gernandt et al. 2018), Table Mountain pine has a fragmented and widespread distribution from Georgia to Pennsylvania (Zobel 1969). Table Mountain pine forests likely expanded during the industrial logging era of the late 19th and early 20th century but have greatly declined since the middle of the 20th century as a result of fire suppression and changing land uses (Williams 1998). According to current forest inventory data, approximately 82,000 ha of forest have at least 50% Table Mountain pine biomass, with more than half of that area in Virginia (Burrill et al. 2018). Because of its decline, it is a species of conservation concern (Jetton et al. 2015) that is increasingly valued by land managers for diversity and as a foundation of an uncommon conifer community in a landscape dominated by hardwood forests (Brose 2017). Although its crooked and irregular form generally limits its commercial use to fuel and pulpwood, it provides ecosystem services, including the prevention of soil erosion and food and cover for wildlife (Della-Bianca 1990).

Fire is a key component in Table Mountain pine regeneration. Trees in most populations have highly serotinous seed cones that are opened by fire, which also exposes mineral soil and eliminates competing vegetation for seedlings (Zobel 1969). Closed cones may contain viable seed for as long as 25

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years, although seed viability declines quickly in cones older than five years (Barden 1979). At the northern end of the range, cones are only weakly serotinous and open without fire (Brose and Waldrop 2010; Brose 2017). In general, Table Mountain pine is thought to require regular canopy-opening disturbances, such as those resulting from fires, for successful regeneration and more regular lower-intensity fires to reduce competition (Williams 1998; Waldrop and Brose 1999; Brose and Waldrop 2010). This may not always be the case, however, especially at extremely xeric sites where regeneration may occur following infrequent and extremely intense fires that remove competing ericaceous shrubs (Barden and Costa 2020). Appalachian pine-oak communities, including those with Table Mountain pine, were likely maintained through positive feedback with flammable vegetation by a regime of frequent surface fires and occasional severe fires (as well as insect infestations and other disturbances) followed by pine recruitment episodes (Aldrich et al. 2010; Lafon et al. 2021). However, the suppression of fires starting early in the 20th century allowed the establishment of competing species, especially hardwoods, that are replacing Appalachian pine species, including Table Mountain pine (Brose 2017; Lafon et al. 2021). Now, the optimal recruitment and maintenance of Table Mountain pine forests is unlikely in the absence of relatively high-intensity fire (Williams and Johnson 1992; Lafon and Kutac 2003).

The current lack of adequate regeneration in Table Mountain pine is acute. An analysis using Forest Inventory and Analysis (FIA) data of the size class distributions of US tree species indicated the ratio of seedlings and saplings to large trees in Table Mountain pine was unsustainable across its distribution and in each of the ecological zones encompassed by its range (Potter and Riitters 2022). This indicates the species may be at risk of genetic variation loss through insufficient regeneration. It may also be at particular risk to future insect and disease threats because of its demographic attributes (Potter et al. 2019). Mortality caused by southern pine beetle (*Dendroctonus frontalis* Zimmermann) in particular can negatively affect the density and basal area of Table Mountain pine stands (Lafon and Kutac 2003; Knebel and Wentworth 2007), with recent and future warmer minimum winter temperatures allowing the insect to move to higher elevations and northerly latitudes, including Pennsylvania (Dodds et al. 2018). At the same time, pine beetle infestation and drought also may increase the frequency of high-intensity fires in Table Mountain pine stands that would assist in their regeneration (Williams 1998; Lafon and Kutac 2003).

Beginning in 2010, the USDA Forest Service and Camcore, an international tree breeding and conservation program at North Carolina State University, cooperated in a gene conservation project that collected seed from 262 mother trees in 38 populations, representing the largest genetic resource of Table Mountain pine outside its natural stands (Jetton et al. 2015). That project additionally included the sampling of leaf material for a range-wide assessment of genetic variation using highly polymorphic microsatellite markers, as well as an evaluation of the conservation effectiveness of seed collections for the species. These analyses build on and update a genetic assessment of 20 Table Mountain pine populations using 21 allozyme loci (Gibson and Hamrick 1991). Specifically, a denser sampling of Table Mountain pine populations using highly polymorphic markers enables a more thorough quantification of genetic variation patterns and structure and of relationships between these metrics and population isolation.

Table Mountain pine occurs in relatively small populations generally isolated from each other, a situation that may result in high between-population variation and low within-population genetic variation (Willi et al. 2006) as a result of the small-population processes of inbreeding and genetic drift (Young et al. 1996). The results in Gibson and Hamrick (1991) indicate that this has occurred in Table Mountain pine. A microsatellite study of genetic variation in Carolina hemlock (*Tsuga caroliniana* Engelm.), another Appalachian conifer characterized by small and isolated populations occurring in rocky high-elevation sites, discovered unexpectedly high levels of differentiation and inbreeding (Potter et al. 2017) given that, as in most other conifers, its pollen and seed are wind dispersed (Godman and Lancaster 1990). Tree species that disperse genetic material over long distances are expected to achieve high-interpopulation gene flow that results in high overall genetic variability and low differentiation among populations (Hamrick et al. 1992; Hamrick and Godt 1996). In fact, that was likely the situation for another Southern Appalachian endemic conifer that exists in a limited number of isolation populations, Fraser fir (*Abies fraseri* [Pursh.] Poir). A microsatellite study found relatively little differentiation among its populations, most likely the result of frequent and effective long-distance pollen dispersal (Potter et al. 2008). We hypothesized that Table Mountain pine exhibits a genetic structure more like that of Carolina hemlock (highly differentiated with levels of differentiation associated with distance among populations) than that of Fraser fir because Table Mountain pine's wind-dispersed pollen is relatively large compared to other pine species (Della-Bianca 1990) and therefore, like Carolina hemlock pollen, may not be well-suited for long-distance dispersal.

Seeds collected recently from Table Mountain pine populations produced seedlings for the restoration of a stand damaged by a tornado (Jetton et al. 2015) and were used in 2020 to establish two Forest Service seed orchards to supply seedlings for species restoration efforts (Camcore 2022). In its genetic sampling efforts across species, Camcore aims to collect seeds from ten to twenty mother trees from each of six to ten populations (Dvorak et al. 1999; Dvorak 2012) with a goal of capturing 95% of genes in target populations occurring at frequencies of 5% or greater (Marshall and Brown 1975). That collection goal was achieved for Table Mountain pine (Jetton et al. 2015). Camcore further aims to deploy seedlings grown from collected seed in conservation plantings encompassing a minimum of ten open-pollinated families of five seedlings from each sampled population, planted on two sites; the objective is to maintain an effective population size of approximately 30 for each population that encompasses most common alleles (those at ≥ 5% frequency) and at least some rare alleles (Dvorak 2012). Meeting the 10-family-per-population goal is not always possible in practice, however, because of factors including seed availability on trees at collection time and the germination and survival of seedlings grown from collected seed. Additionally, the establishment and long-term maintenance of a conservation planting require large investments in personnel, materials, nursery space, and land, but such resources are often limited. It is necessary, therefore, to assess how best to represent as much diversity as possible using the smallest number of open-pollinated families and to evaluate the importance of a sampling strategy that represents both family and population structure. Computer simulations can be used to test such population genetics sampling schemes (Hoban et al. 2012; Hoban et al. 2013).

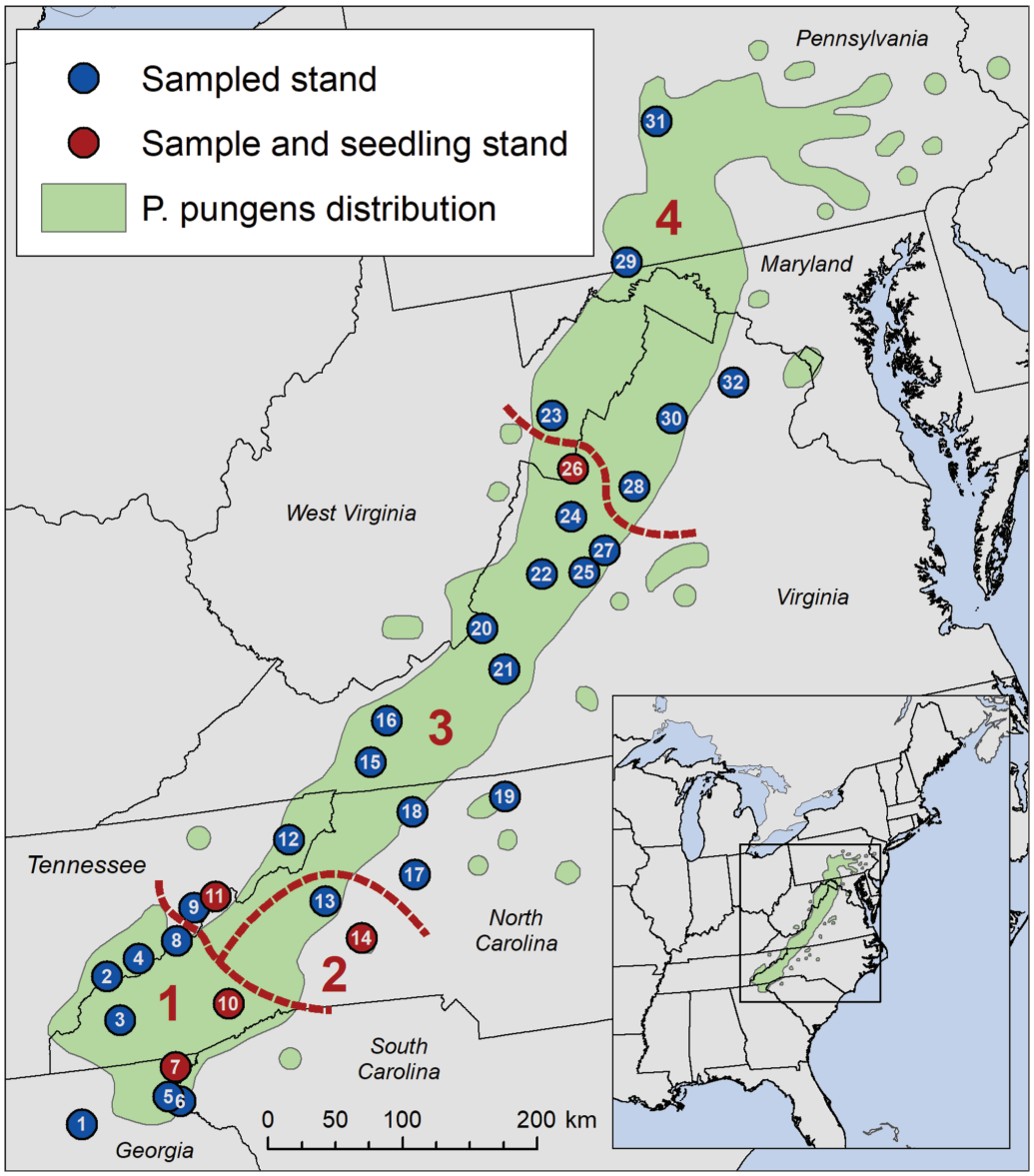

Figure 1 Sampled populations of Table Mountain pine (*Pinus pungens*) with the four seed collection zones delineated for the species. Populations 7, 10, 11, 14, and 26 are those from which both foliage and seeds were collected. See Table 1 for population information.

Patterns of genetic variation in Table Mountain pine, as well as their causes, have important implications for the genetic conservation of this species. In this study, we used seven highly polymorphic microsatellite molecular markers to assess genetic structure and variation across 26 populations of Table Mountain pine and within a subset of seeds collected from five of those populations. Our specific objectives were to (1) assess the relationship between the isolation and environmental attributes of populations and their genetic diversity and differentiation, (2) evaluate differences in genetic variation among seed collection zones established for the species, and (3) assess the genetic consequences of varying strategies for the deployment of collected seed in conservation plantings.

Materials and Methods

Sample Collection and DNA Extraction

We sampled 32 populations of Table Mountain pine throughout the geographic range of the species in spring of 2014, encompassing 346 trees (Figure 1; Table 1). Four 6-inch branch tips (one from each cardinal direction on the tree) were cut and bagged from each tree. Where possible, at least ten trees per population were sampled while maintaining a distance of at least 100 m between sampled trees to reduce the likelihood of sampling neighbors, consistent with established gene conservation strategies used by Camcore in Table Mountain pine seed collections (Jetton et al. 2015). The distance between sampled trees was maintained because neighboring trees are more likely to be closely related as a result of short-distance seed dispersal (Brown and Hardner 2000). As a result, some populations encompassed smaller samples than desired, but this was unavoidable given the rarity of the species and the small sizes of most populations. Six populations encompassing fewer than nine sampled trees were excluded from population-level analyses. We note, however, that the sample sizes of populations in the study generally reflect the relative sizes of the stands, and we would not have been able to sample more

Table 1. Identification number, location, sample size, coordinates, elevation, seed collection zone, and climate values for the populations sampled for the microsatellite analysis of Table Mountain pine.

						Elev.		Nearest	Temperature (°C)		Precipitation
ID	Population	County, State	N	Latitude	Longitude	(m)	Zone	(km)	Min.	Max.	Mean (mm)
1	Camp Merrill	Lumpkin, GA	1	34.63	-84.12	606	1	68.0	6.2	19.1	1671
2	Cades Cove	Blount, TN	5	35.56	-83.83	851	1	27.1	4.7	17.7	1592
3	Nolton Ridge	Graham, NC	10	35.29	-83.70	1097	1	33.6	4.6	18.3	1667
4	Cherokee Orchard	Swain, TN	13	35.68	-83.48	1534	1	27.1	4.6	18.1	1461
5	Tallulah Gorge	Rabun, GA	10	34.74	-83.39	445	1	9.6	7.2	20.5	1675
6	Pine Mountain	Oconee, SC	9	34.70	-83.30	507	1	9.6	8.3	21.6	1519
7	Walnut Fork*	Rabun, GA	20	34.92	-83.28	702	1	22.9	5.8	19.2	1850
8	Poor Mountain SC	Oconee, SC	12	34.77	-83.14	479	1	27.8	7.8	21.0	1615
9	Meadow Creek	Cocke, TN	12	35.97	-82.96	739	3	18.3	5.9	19.2	1157
10	Looking Glass Rock*	Transylvania, NC	20	35.30	-82.79	1186	1	60.8	4.7	17.4	1740
11	Greene Mountain*	Greene, TN	20	36.03	-82.77	726	3	18.3	4.9	18.0	1191
12	Iron Mountain	Johnson, TN	12	36.33	-82.10	883	3	53.4	5.1	18.3	1230
13	Table Rock Mountain	Burke, NC	13	35.89	-81.88	1181	2	38.5	4.7	17.5	1424
14	South Mountains*	Burke, NC	10	35.60	-81.61	677	2	38.5	7.3	20.4	1319
15	Snake Den Mountain	Smyth, VA	10	36.76	-81.34	1064	3	33.2	3.7	16.3	1180
16	Little Walker	Wythe, VA	9	37.01	-81.18	751	3	33.2	3.8	16.7	1053
17	Rocky Face	Alexander, NC	6	35.97	-81.11	536	3	46.9	6.8	20.5	1247
18	Stone Mountain	Wilkes/Alleghany, NC	12	36.39	-81.04	680	3	46.9	5.4	19.1	1206
19	Hanging Rock	Stokes, NC	11	36.40	-80.26	648	3	70.5	6.6	19.2	1246
20	Potts Mountain	Craig, VA	13	37.53	-80.21	600	3	34.3	4.9	18.4	979
21	Poor Mountain VA	Roanoke, VA	10	37.23	-80.09	673	3	34.3	5.3	18.1	1054
22	North Mountain	Rockbridge, VA	12	37.82	-79.63	927	3	32.0	4.4	17.2	1110
23	Smoke Hole	Pendelton, WV	5	38.85	-79.31	877	4	42.4	4.9	18.5	965
24	Elliott Knob	Augusta, VA	10	38.16	-79.31	1203	3	35.4	3.3	15.6	1142
25	Buena Vista	Rockbridge, VA	10	37.79	-79.27	748	3	22.3	4.7	17.2	1148
26	Briery Branch*	Rockingham, VA	14	38.48	-79.22	1133	3	35.6	2.4	14.2	1149
27	Bald Mountain	Nelson, VA	7	37.90	-79.05	867	3	22.3	4.1	16.2	1202
28	Shenandoah South	Appomattox/Albemarle/Madison, VA	10	38.74	-78.31	1110	4	47.9	6.8	19.2	1136
29	Buchanan	Bedford, PA	5	39.77	-78.43	371	4	107.0	3.8	16.3	955
30	Shenandoah North	Warren/Rappahannock/Madison, VA	10	38.69	-78.30	876	4	53.6	3.3	14.2	1230
31	Stone Valley	Huntingdon, PA	10	40.66	-77.95	351	4	107.0	4.2	15.7	974
32	Bull Run	Fauquier, VA	15	38.85	-77.72	412	4	53.6	6.0	18.1	1045

*Seed collection populations.

trees from these populations while maintaining the required distances between sampled trees.

Using seeds collected between 2010 and 2014 (Jetton et al. 2015), we grew seedlings from five Table Mountain pine populations to assess the efficacy and efficiency of *ex situ* seed conservation efforts for the species. Specifically, we aimed to grow at least ten seedlings from each of ten mother trees (i.e., half-sibling open-pollinated families) from each of the five populations. The populations were selected based on the availability of seeds from at least ten mother trees and to approximately represent each of four seed collection zones developed for the species based on a cluster analysis of climate similarity (Jetton et al. 2015) (Figure 1). In summer 2015, following a 24-hour cold-moist stratification, 20 seeds per mother tree were sown into Ray Leach super cells using a soil medium that was three parts composted pine bark, one part perlite, and one part coarse sand. Approximately four months after germination, about 50 mg of fresh leaf tissue was harvested from 509 seedlings. At the end of this process, only one population (Briery Branch) encompassed ten families with at least five seedlings, whereas two populations had nine such families (Greene Mountain and Walnut Fork), one population had eight (South Mountains), and one population had six (Looking Glass Rock).

The 855 tree and seedling foliage samples were kept in cold storage until they were sent to the National Forest Genetics Laboratory (NFGEL) in Placerville, CA, for DNA extraction and microsatellite analysis. Genomic DNA for all samples was extracted from the needle samples using the DNEasy 96 Plant Kit (Qiagen, Chatsworth, CA). DNA concentrations were determined using a Gemini XPS Microplate Spectrofluorometer (Molecular Devices, Sunnyvale, CA) with PicoGreen dsReagent (Invitrogen, Carlsbad, CA).

Microsatellite Genotyping and Analysis

Range-wide analyses using codominant and highly polymorphic microsatellite markers (Kalia et al. 2011) can be useful for guiding genetic conservation efforts and understanding recent population processes (e.g., Potter et al. 2015; Ony et al. 2021). A set of 149 4-, 5- and 6-mer microsatellite primer pairs mined from the loblolly pine (*Pinus taeda* L.) RefSeq release 0.6 genome (Zimin et al. 2014) was screened for use in Table Mountain pine using DNA isolated from a set of 17 trees of the species. 51 primer pairs were amplified in a preliminary panel of Table Mountain pine samples, of which 24 were not polymorphic and three had generally poor or no amplification. The remaining 24 primer pairs were rated for consistency and quality of amplification, with the best eight sent to NFGEL. One of these did not amplify well there, so a final set of seven informative, consistently amplifying, and easy-to-score markers were used for the study (Table 2).

Polymerase chain reaction (PCR) amplification was performed in 10 µL reaction volumes containing 10 ng genomic DNA, 1 mM each dNTP, 10X Taq buffer, 5.0 mM $MgCl_2$, and 0.5 units of HotStarTaq DNA polymerase. The PCR protocol was 2 min at 94°C; 20 cycles of 30 s at 94°C (denaturation), 30 s at 65°C for the first cycle minus 0.5°C each subsequent cycle (annealing), and 1 min at 72°C (extension); and 25 cycles of 30 s at 92°C, 30 s at 55°C, and 1.5 min at 72°C; all followed by a final 15 min extension at 72°C and an indefinite hold at 4°C. The PCR products for all markers were separated on an ABI Prism 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA), as recommended by the manufacturer. Peaks were sized and binned, and then alleles were called using GeneMarker (SoftGenetics, State College, PA), with GeneScan ROX as an internal size standard for each sample. Visual checks were performed on all peaks.

Genetic Variation Analyses

We estimated inbreeding coefficients (F_{IS}) and null allele frequencies for each of the seven loci using the population inbreeding model–based approach of Chybicki and Burczyk (2009), an expectation-maximization algorithm in the program INest 2.0 that takes into account the potential presence of inbreeding within a sample population. We ran the "exclusion null alleles" method in FreeNA (Chapuis and Estoup 2007) with 50,000 replicates to calculate among population differentiation (F_{ST}) across loci, overall, and between all pairs of natural and seedling populations, while accounting for estimated null alleles, and to generate a matrix of population-pairwise Cavalli-Sforza chord distances (D_C) (Cavalli-Sforza and Edwards 1967) that also accounted for estimated null alleles. To further assess the partitioning of diversity among and within populations, we conducted an analysis of molecular variance (AMOVA) (Excoffier et al. 1992; Huff et al. 1993) using GenAlEx 6.41 (Peakall and Smouse 2012), with the significance of the variance components determined with 999 permutations. The AMOVA method generates Φ_{PT}, which estimates the proportion of the total variance partitioned among populations (Excoffier et al. 1992; Huff 1997) and is analogous to F_{ST} (Peakall and Smouse 2006). Using GENEPOP 4.2 (Raymond and Rousset 1995), we estimated inter-population gene flow (N_m) with the private allele method, corrected for sample size, and performed Fisher's exact tests for Hardy-Weinberg equilibrium (HWE) for each locus and population, based on one hundred batches and one thousand iterations. We then employed the MULTTEST procedure in SAS 9.4 (SAS Institute Inc. 2013) to calculate *q*-values (*p*-values adjusted for the false discovery rate associated with multiple comparisons) for each HWE test. FSTAT 2.3.9.2 (Goudet 2002) tested for linkage disequilibrium between pairs of loci based on 1,560 permutations and adjusted for multiple comparisons.

We also generated a suite of population-level statistics for both natural populations encompassing at least nine sampled trees and seedling populations. GenAlEx 6.41 (Peakall and Smouse 2012) calculated percent polymorphic loci (P_P), mean observed (H_O) and expected (H_E) heterozygosities across loci, and fixation index (F) across loci, calculated as $(H_E–H_O)/H_E$ for each locus, with substantial positive values indicating inbreeding and substantial negative values indicating excess of heterozygosity. It also generated the mean alleles per locus (A), a list of unique (private) alleles (A_U) present in each population, and the mean number across loci of locally common alleles (frequency>5%) found in 25% or fewer populations. We used the package HP-Rare (Kalinowski 2005) to account for unequal population sample sizes and produce unbiased population-level estimates of the mean number of

Table 2. Description of the seven nuclear microsatellite loci used in the study, with size range, measures of genetic variation, inbreeding, deviation from Hardy-Weinberg equilibrium, and estimated null allele frequency for each, using data from the 346 trees sampled from 32 natural stands.

Locus	Size range	*A*	H_O	H_E	A_E	F_{ST}	F_{IS}	HWE	Null
PtSIFG_5230	346–358	3	0.318	0.327	1.49	0.093	0.030	*	0.000
PtSIFG_5307	174–218	11	0.613	0.635	2.74	0.017	0.037	ns	0.000
PtSIFG_5312	291–307	6	0.506	0.715	3.51	0.037	0.294	*	0.099
PtSIFG_5326	276–330	13	0.754	0.801	5.03	0.028	0.061	*	0.000
PtSIFG_5334	231–257	9	0.335	0.595	2.47	0.048	0.437	*	0.124
PtSIFG_5339	222–260	12	0.653	0.789	4.74	0.028	0.174	*	0.049
PtSIFG_6065	208–226	7	0.332	0.722	3.60	0.056	0.541	*	0.193
Total		61				0.039		*	
Mean		8.71	0.502	0.655	3.37	0.044	0.225		0.066

A, alleles per locus; *HO*, observed heterozygosity; *HE*, expected heterozygosity; A_E, effective alleles; F_{ST}, among-population variation; F_{IS}, inbreeding coefficient; HWE, Hardy-Weinberg exact test of heterozygote deficiency, with * $q < 0.05$ using false discovery rate adjustment; Null, estimated proportion of null alleles (Chybicki and Burczyk 2009).

private alleles (A_{UR}) and alleles per locus (A_R) using rarefaction (standardized to 20 genes per population for the natural stands and for the seedling populations).

We were interested in the degree to which population-level diversity estimates differ among populations in different seed collection zones and between populations both proximal and highly isolated. The seed collection zones were determined for the species based on a cluster analysis of climate similarity using 19 bioclimatic variables from WorldClim (Jetton et al. 2015) in the absence of resources and time to establish a common garden experiment to inform seed zone delineation based on phenotypic differences. To ensure an adequate number of populations for statistical analyses, we combined the two populations in seed zone 2 with the seven in neighboring seed zone 1 (southern seed zones), and we combined the four populations in seed zone 4 with the 13 in seed zone 3 (northern seed zones). Populations were classified as more isolated if the distance to the nearest sampled population was more than the mean distance of all populations to their nearest neighbors (41 km). We acknowledge this threshold is arbitrary but maintain that it is reasonable given the ability of pines to disperse pollen across large distances (Williams 2017) and the small amount of gene flow needed to avert genetic drift and inbreeding in isolated plant populations (Ellstrand 1992). Within these groups of populations (by seed zones and by more isolated or less isolated), we calculated the means for the following metrics: A_R, A_{UR}, P_P, H_O, H_E, F, mean pairwise D_C with all other populations, and mean pairwise F_{ST} with all other populations. To test the null hypothesis that there was no significant difference within each set of groups, we conducted a set of multiple sample, nonparametric Kruskal-Wallis tests using the NPAR1WAY procedure in SAS 9.4 (SAS Institute Inc. 2013), with 10,000 Monte Carlo runs generating *p*-values. We then employed the MULTTEST procedure to calculate *q*-values as described above. Finally, we used the *vegan* package (Oksanen et al. 2020) in R version 4.02 (R Core Team 2022) to conduct a Mantel test of correlation between pairs of population-level dissimilarity matrices, specifically between each of a set of genetic variation and differentiation metrics (differences in A_R, A_u, H_O, H_E, and F, and pairwise D_C and F_{ST}) and pairwise geographic distance.

Genetic Structure Analyses

We ran STRUCTURE 2.3.4 (Pritchard et al. 2000), a model-based Bayesian clustering method, to estimate the number and composition of genetic clusters that best describe the population structure within Table Mountain pine. We used the admixture model with a burn in of 50,000 replicates and, subsequently, 500,000 Markov chain Monte Carlo (MCMC) replicates. We ran ten iterations for each possible maximum number of genetic clusters (K) from 1 to 10. We then calculated the ΔK statistic of Evanno et al. (2005) using STRUCTURE HARVESTER (Earl and Vonholdt 2012), with the ΔK statistic revealing a dominant peak at K = 6. CLUMPP 1.1.2 (Jakobsson and Rosenberg 2007) generated averaged Q matrices of individual and population posterior cluster probabilities using the greedy algorithm and the G' pairwise matrix similarity statistic. Finally, we generated maps showing the geographic distribution of the K = 6 clusters throughout the range of Table Mountain pine after importing the population matrix into ArcMap 10.7.1(ESRI 2019).

We used discriminant analysis of principal components (DAPC) to estimate the number of genetic clusters in the microsatellite data because STRUCTURE assumes HWE within populations (Putman and Carbone 2014) and because our exact tests for HWE indicated a significant deficit of heterozygotes for all loci. DAPC does not make assumptions about HWE because it does not rely on a particular population genetics model (Jombart et al. 2010). Using the *adegenet* package (Jombart 2008), version 2.0.1, in R version 4.02 (R Core Team 2022), we applied K-means clustering of K from 1 to 30 after transforming the raw allelic data with a principal component analysis (PCA). We inspected Bayesian information criterion (BIC) values for each K to determine which number of clusters best maximized variation between clusters and minimized variation within them. The BIC values declined sharply from K = 1 to K = 10, with a slight "elbow" at about K = 6, followed by a flat trend until K = 13 and then increasing. Selecting K = 6, we conducted a discriminant analysis to determine individual sample membership probabilities within each of the clusters, and then calculated the proportion of overall genetic cluster presence probability for each population. We used ArcMap 10.7.1 (ESRI 2019) to depict the geographic distribution of the clusters within each of the sampled stands.

Sample Collection Simulations

We conducted a series of simulations to assess the potential effectiveness of different sampling approaches in representing genetic variation in potential *ex situ* conservation plantings. These simulations used the microsatellite marker data of 498 seedlings (those from the 509 total seedlings for which we had no missing data) grown from seed collected at five of the natural stands across the distribution of Table Mountain pine. The seedlings were grown in 2015 from seed collected from Briery Branch, Virginia; Greene Mountain, Tennessee; Looking Glass Rock, North Carolina; South Mountains, North Carolina; and Walnut Fork, Georgia (Table 1, Figure 1).

In the first of these sets of simulations, we tested how well the random selection of between five and nine or ten five-seedling open-pollinated families would represent genetic variation within three populations. We used the SURVEYSELECT procedure in SAS 9.4 (SAS Institute Inc. 2013) to simulate the random collection of five seedlings from five to nine randomly selected families from the Greene Mountain and Walnut Fork populations and from five to ten randomly selected families from the Briery Branch population. (These represented the maximum number of five-seedling families available for each population). The simulated collections were conducted 100 times for each of the population X family number combinations. We calculated the allelic richness, H_O, H_E, and F of the seedlings from each simulated sample collection. We then determined the mean value of the genetic metrics of the 100 sample collections within each of the sampling combinations. To test the null hypothesis that there were no significant differences in genetic metrics among the number of families sampled, we conducted multiple-sample, nonparametric Kruskal-Wallis tests for each population using the NPAR1WAY procedure in SAS 9.4 (SAS Institute Inc. 2013) in which *p*-values were generated by 10,000 Monte Carlo runs. To test whether these samples meet Camcore's objective of encompassing most common alleles and some rare alleles in its conservation plantings (Dvorak 2012), we calculated the mean number of alleles of ≥ 5% frequency and of < 5% frequency included in each set of collections.

In the second of these simulation sets, we assessed the effectiveness of a sampling strategy based on both open-pollinated family and population structure versus one based only on family structure. We again used the SURVEYSELECT procedure in SAS 9.4 (SAS Institute Inc. 2013) to generate simulated collections, this time of 125 seedlings. The sampling was done in three ways: (1) five seedlings at random from 25 families also at random, (2) five seedlings at random from five families at random from each of the five populations, and (3) 125 seedlings at random across populations and families. (The samples in each, 125, was chosen to keep the number constant across sampling methods rather than to meet a specific gene conservation objective.) These simulated collections were completed 100 times for each of the three groups. Mean genetic diversity metrics calculated and tests of differences among the groups were conducted in SAS as described above, as was the calculation of the percent of common and rare alleles represented.

Results

Species-Level Microsatellite Results

The mean number of alleles per locus was 8.71 across the 346 trees sampled from natural stands (total alleles = 61), ranging from a minimum of three alleles for *PtSIFG_5230* to a maximum of 13 for *PtSIFG_5326* (Table 2). Mean expected heterozygosity (H_E) across loci (0.655) was higher than mean observed heterozygosity (H_O) (0.502), while exact tests for HWE indicated a significant deficit of heterozygotes for all but one locus (*PtSIFG_5307*). The mean positive inbreeding coefficient (F_{IS}) of 0.225 indicated a deficit of heterozygotes and the presence of inbreeding. The average estimated proportion of null alleles across loci was 0.066. We did not detect linkage disequilibrium between any pairs of loci at a *p*-value adjusted for multiple comparisons.

F_{ST} values did not differ when adjusted for null alleles compared to not adjusted (F_{ST} across loci adjusted for nulls = 0.039, 95% confidence interval: 0.029–0.056; F_{ST} across loci, not adjusted for nulls = 0.037, 95% confidence interval: 0.027–0.052). When adjusting for estimated null alleles, individual locus F_{ST} values ranged from 0.017 (*PtSIFG_5307*) to 0.093 *(PtSIFG_5230)* (Table 2), with a mean of 0.044. The AMOVA results similarly indicated that a relatively low level of microsatellite variance (Φ_{PT} = 0.056) was partitioned among populations, with about 94% occurring within populations (Supplementary Table S1). Interpopulation gene flow (*Nm*) was estimated at 4.27, or approximately four per generation.

For the 498 seedlings with complete genotypic data, the total alleles sampled (61) and the alleles per locus (8.71) were the same as for the trees (Supplementary Table S2), but the alleles were not identical between the groups. Specifically, 11 alleles present in the tree sample were not in the seedling sample and vice versa. The mean H_E across loci for seedlings (0.659, standard error 0.060) was comparable to the mean H_E for mature trees (0.655, standard error 0.062), but the seedling H_O was lower than that for trees (0.465 compared to 0.502), although the estimates were within each other's standard errors (0.061 and 0.067, respectively). The mean F_{IS} was higher across loci for seedlings (0.291) than for mature trees (0.225), although the standard error bars (0.069 and 0.078, respectively) overlapped for the estimates. The F_{ST} was higher for seedlings (0.060 versus 0.037) and its 95% confidence interval only slightly overlapped with the interval for trees (0.045–0.079 versus 0.027–0.052).

Population-Level Genetic Variation and Differentiation

The 26 Table Mountain pine populations encompassing at least nine sampled trees averaged 4.60 alleles per locus (*A*) and 4.43 alleles per locus standardized for sample size (A_R) (Table 3). For most populations, 100% of the loci were polymorphic. Eight populations contained a single unique allele (A_U), and one population (South Mountains, #14) had two. Populations with higher A_R and unique alleles were distributed across the species distribution (Figure 2A and 2B). On average, populations had a 0.412 frequency of locally common alleles (>5% frequency) that were globally rare (found in ≤ 25% of populations) (Table 3). Three populations in Tennessee (Meadow Creek [#9], Greene Mountain [#11], and Iron Mountain [#12]) had particularly high frequencies of locally common/globally rare alleles, along with Shenandoah South (#28) in Virginia. Most populations were inbred (Figure 2C) and significantly out of HWE (Table 3). Most genetically differentiated populations, based on mean pairwise F_{ST} between each population and the 25 others (Table 3, Figure 2D), were located in the southern and northern extremities of the Table Mountain pine distribution. Mean pairwise F_{ST} averaged across populations was 0.028 and the mean pairwise chord distance (D_C) was 0.258.

Between 67 and 141 seedlings were grown from mother trees in each of five stands (Walnut Creek, Looking Glass Rock, Greene Mountain, South Mountains, and Briery Branch). A_R varied from 4.36 at Looking Glass Rock to 4.87 at Briery Branch (Supplementary Table S3). Briery Branch had the most unique alleles (8), whereas Walnut Fork and Looking Glass Rock had 1 and 0, respectively. H_E exceeded H_O for all seedling populations; mean H_O was less than for the natural stands (0.462 versus 0.495). All seedling populations were inbred, on average (F = 0.249) more so than the trees in the natural stands (F = 0.107).

Genetic Structure Analyses

We found no clear association between geography and genetic clusters. Based on the ΔK method (Evanno et al. 2005), the STRUCTURE analysis most strongly supported the possibility of six genetic clusters in Table Mountain pine (Supplementary Figure S1). There was no strong association between genetic clusters and any parts of the species range (Figure 3a). Three clusters (1, 3, and 5) tended to be more prevalent in the southern half of the distribution, and cluster 2 was more northern. Two others (clusters 4 and 6) were relatively evenly distributed geographically. The *adegenet* clustering analysis indicated the samples could be grouped into between about six and 14 clusters; we selected the smallest number. When mapped, these *adegenet* clusters were also not closely aligned with any region, although two (clusters 2 and 6) were generally more southern and one (cluster 4) was more northern.

Group Comparisons and Correlations with Geographic Variables

We detected some marginally significant differences in measures of genetic variation and differentiation between northern and southern Table Mountain pine seed collection zones and between more and less isolated populations. Allelic richness (A_R)

Table 3. Measures of genetic variation for each of 26 natural stands of Table Mountain pine containing at least nine sampled trees, based on seven nuclear microsatellite loci.

ID	Population	A	A_R	A_U	A_{UR}	P_P (%)	A_{25}	H_O	H_E	F	HWE	Mean D_C	Mean F_{ST}
3	Nolton Ridge	3.71	3.71	0	0	100.0	0.00	0.514	0.568	0.086	ns	0.275	0.044
4	Cherokee Orchard	5.14	4.72	0	0.03	100.0	0.43	0.516	0.593	0.079	*	0.261	0.042
5	Tallulah Gorge	4.29	4.29	0	0	100.0	0.29	0.500	0.573	0.121	ns	0.251	0.029
6	Pine Mountain	4.43	4.43	0	0	100.0	0.29	0.476	0.638	0.252	*	0.257	0.011
7	Walnut Fork	4.86	4.47	1	0.11	100.0	0.43	0.579	0.660	0.121	*	0.259	0.035
8	Poor Mountain SC	4.43	4.24	0	0	100.0	0.57	0.560	0.585	0.044	ns	0.275	0.041
9	Meadow Creek	4.71	4.54	0	0	100.0	0.86	0.512	0.624	0.130	*	0.267	0.028
10	Looking Glass Rock	4.71	4	0	0	100.0	0.14	0.393	0.510	0.225	*	0.251	0.056
11	Greene Mountain	5.14	4.43	0	0	100.0	0.71	0.493	0.603	0.152	*	0.231	0.024
12	Iron Mountain	4.71	4.46	1	0.12	100.0	0.71	0.500	0.599	0.152	*	0.251	0.019
13	Table Rock Mountain	4.86	4.56	1	0.14	100.0	0.43	0.516	0.601	0.094	*	0.272	0.054
14	South Mountains	4.29	4.29	2	0.32	100.0	0.29	0.440	0.619	0.300	*	0.277	0.016
15	Snake Den Mountain	4.71	4.71	0	0	100.0	0.57	0.600	0.610	-0.007	ns	0.256	0.021
16	Little Walker	4.71	4.71	1	0.15	100.0	0.14	0.508	0.653	0.249	*	0.265	0.011
18	Stone Mountain	4.86	4.72	1	0.14	100.0	0.57	0.500	0.631	0.214	*	0.252	0.020
19	Hanging Rock	4.71	4.59	0	0	100.0	0.57	0.506	0.586	0.132	*	0.248	0.026
20	Potts Mountain	4.29	4.12	0	0	100.0	0.14	0.451	0.572	0.227	*	0.253	0.038
21	Poor Mountain VA	4.14	4.14	0	0.02	85.7	0.29	0.429	0.538	0.224	*	0.271	0.029
22	North Mountain	4.57	4.42	1	0.12	100.0	0.14	0.524	0.668	0.204	*	0.241	0.014
24	Elliott Knob	4.57	4.57	0	0	100.0	0.57	0.529	0.641	0.142	*	0.262	0.023
25	Buena Vista	4.29	4.29	0	0	100.0	0.29	0.571	0.595	0.071	ns	0.257	0.013
26	Briery Branch	5.00	4.72	1	0.1	100.0	0.43	0.531	0.630	0.130	*	0.232	0.019
28	Shenandoah South	4.57	4.57	0	0	100.0	0.71	0.500	0.621	0.162	*	0.258	0.020
30	Shenandoah North	4.57	4.57	0	0	100.0	0.57	0.471	0.604	0.193	*	0.272	0.036
31	Stone Valley	4.14	4.14	0	0	85.7	0.14	0.457	0.514	0.099	ns	0.281	0.042
32	Bull Run	5.14	4.75	1	0.1	100.0	0.43	0.486	0.634	0.202	*	0.244	0.023
	Mean	*4.60*	*4.43*	*0.4*	*0.05*	*98.9*	*0.412*	*0.502*	*0.603*	*0.154*	*	*0.258*	*0.028*

A, mean alleles per locus; A_R, mean alleles per locus standardized by rarefaction; A_U, unique (private) alleles; A_{UR}, unique alleles standardized by rarefaction; P_P, percent of polymorphic loci; A_{25}, number of locally common alleles (frequency>5%) found in 25% or fewer populations; H_O, mean observed heterozygosity; H_E, mean expected heterozygosity; F, mean fixation index across loci; HWE, Hardy-Weinberg exact test of heterozygote deficiency, *q < 0.05 using false discovery rate adjustment; Mean Dc, mean pairwise chord distance (Cavalli-Sforza and Edwards 1967) with all other populations; Mean F_{ST}, mean pairwise differentiation with all other populations.

was slightly higher in the northern seed zones (seed zones 3/4) than in the southern seed zones (seed zones 1/2), whereas mean pairwise genetic distance (D_C) and pairwise F_{ST} were higher in the southern seed zones than in the northern ones. These differences, however, were not significant when applying the more conservative q-value to account for multiple comparisons (Table 4). More isolated populations had significantly lower observed heterozygosities (H_O) than less isolated ones, although this again was not significant when accounting for multiple comparisons.

Mantel correlation tests between pairs of population-level genetic and geographic distance matrices revealed significantly positive but weak relationships ($r = 0.225$ and $r = 0.208$) between interpopulation geographic distances and both the pairwise F_{ST} and D_C between populations (Table 5), at $\alpha \leq 0.1$ for both the q-value and p-value used to account for multiple comparisons.

Sample Collection Simulations

When we assessed the effectiveness of sampling approaches in representing genetic variation in potential *ex situ* conservation plantings, we found that more families (and more samples) on average resulted in significantly greater representation of the total number of alleles present in all three populations, as determined by a multiple-sample Kruskal-Wallis test of group differences (Table 6). (As noted above, these families approximately represent the seed collection zones developed for the species based on a cluster analysis of climate similarity.) Ten families in the Briery Branch population on average represented 83.6% of the 52 total alleles across 100 sample draws, whereas nine families from Greene Mountain and Walnut Fork on average sampled 90.5% of 42 and 93.0% of 41 total alleles, respectively. Sampling five families resulted in between 6.6% and 9.2% fewer alleles sampled for the three populations. Common alleles were well represented regardless of the number of families sampled, with a minimum of 94.8% to 97.5% represented for five families. The sampling of rare alleles was less complete, with a maximum of 52.7% to 71.2% and a minimum of 30.8% to 49.4% represented. Expected heterozygosity (H_E) and fixation index (F) were significantly higher when more families were sampled, which is not surprising given that the calculation of these metrics depends in part on allelic richness. H_O, however, did not differ based on number of families sampled.

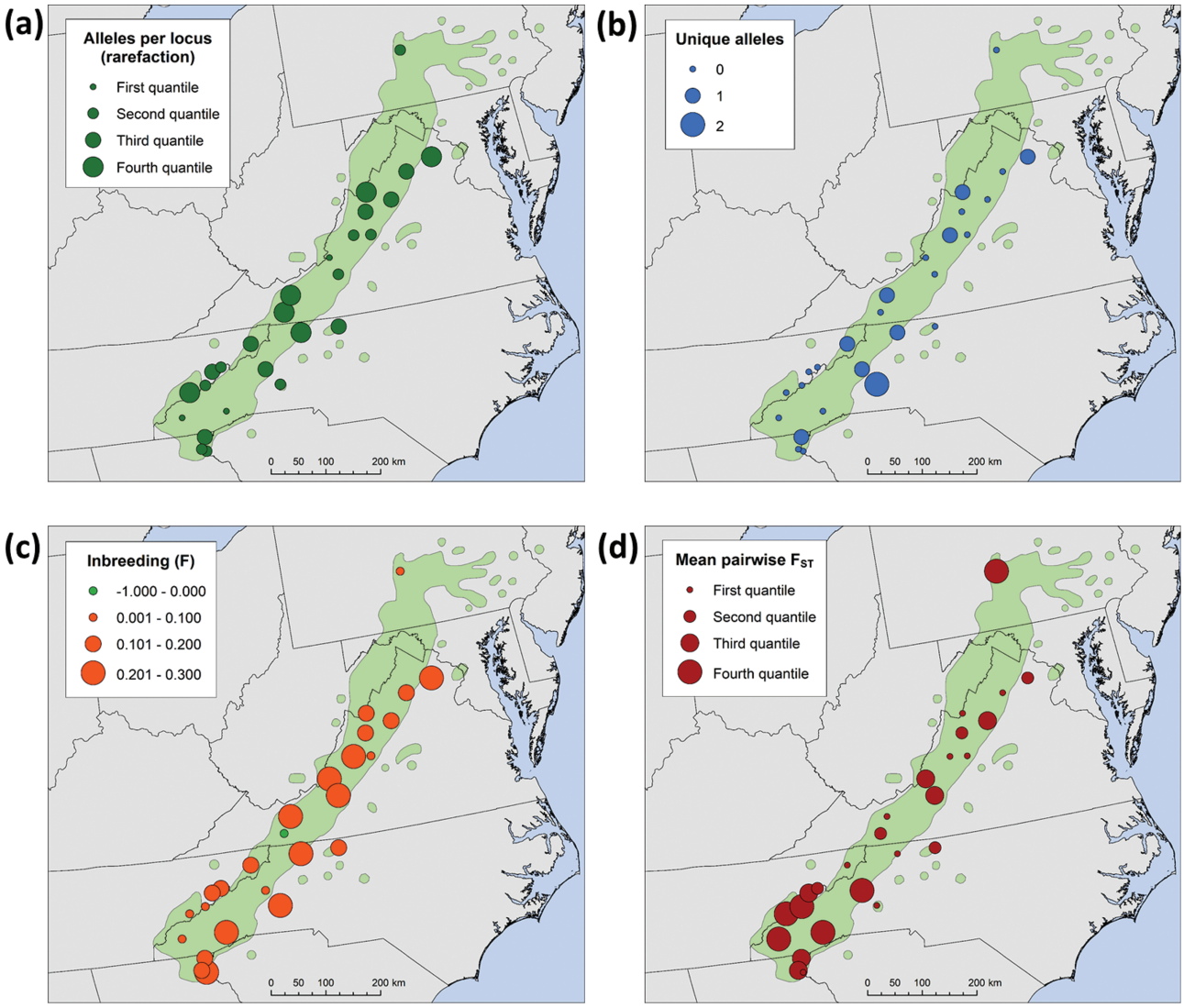

Figure 2 Table Mountain pine population classifications of (a) alleles per locus, rarefied (A), (b) unique alleles (A_U), (c) inbreeding coefficient (F_{IS}), and (d) mean pairwise genetic differentiation (F_{ST}), based on seven polymorphic nuclear microsatellite loci. Only populations with at least nine sampled trees were included.

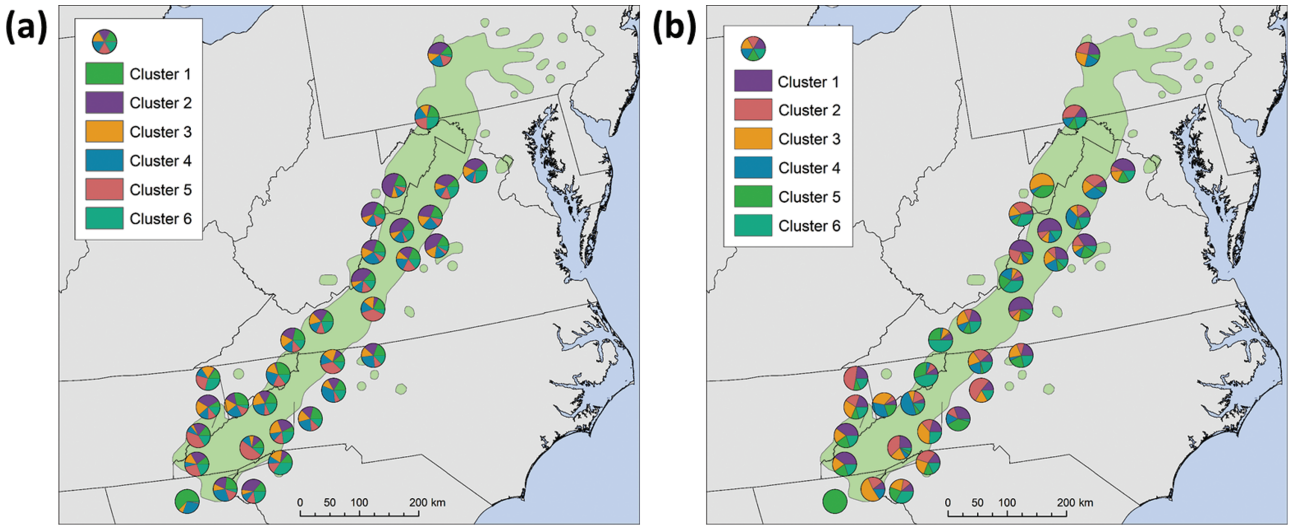

Figure 3 The proportion, within each Table Mountain pine population, of inferred ancestry from K = 6 genetic clusters inferred using (a) Structure 2.3.3 (Pritchard et al. 2000) and (b) discriminant analysis of principal components (DAPC) (Putman and Carbone 2014) in *adegenet* (Jombart 2008). See Table 1 for population information.

Table 4. Comparison between means of genetic variation statistics for populations (encompassing at least nine sampled trees) among seed zones and between populations that are and are not classified as isolated. Values are bold when *p* or *q* was significant at $\alpha \leq 0.1$ from a multiple sample Kruskal-Wallis test, with *q* the false discovery rate adjusted *p*-value.

					Differences	
	Mean	SD	Mean	SD	*p*	*q*
Seed zone	Zone 1/2 (n = 9)		Zone 3/4 (n = 17)			
Allelic richness (A_R)	**4.3**	0.302	**4.5**	0.214	**0.05**	0.16
Unique alleles (A_U)	0.44	0.726	0.35	0.493	0.47	0.47
Percent loci polymorphic (P_P)	100	0	98.3	0.039	0.412	0.47
Observed heterozygosity (H_O)	0.499	0.057	0.504	0.047	0.45	0.47
Expected heterozygosity (H_E)	0.594	0.044	0.607	0.042	0.153	0.306
Inbreeding (*F*)	0.147	0.089	0.157	0.065	0.223	0.357
Mean pairwise D_C	**0.264**	0.011	**0.255**	0.014	**0.06**	0.16
Mean pairwise F_{ST}	**0.036**	0.016	**0.024**	0.009	**0.02**	0.152
Isolation	Less isolated (n = 18)		More isolated (n = 8)			
Allelic richness (A_R)	4.41	0.261	4.48	0.269	0.173	0.371
Unique alleles (A_U)	0.39	0.608	0.38	0.517	0.586	0.586
Percent loci polymorphic (P_P)	99.2	0.034	98.2	0.051	0.533	0.586
Observed heterozygosity (H_O)	**0.513**	0.046	**0.477**	0.038	**0.01**	**0.096**
Expected heterozygosity (H_E)	0.61	0.035	0.587	0.049	0.232	0.371
Inbreeding (*F*)	0.146	0.082	0.172	0.044	0.163	0.371
Mean pairwise D_C	0.259	0.014	0.257	0.013	0.217	0.371
Mean pairwise F_{ST}	0.027	0.013	0.03	0.013	0.332	0.443

Table 5. Results of Mantel tests of correlation between pairs of population-level genetic dissimilarity matrices, specifically of genetic diversity and differentiation and pairwise geographic distance. Values significant at $\alpha \leq 0.1$ are in bold.

	Distance		
	r	*p*	*q*
Allelic richness (A_R)	0.086	0.149	0.348
Unique alleles (A_U)	-0.100	0.957	0.957
Observed heterozygosity (H_O)	-0.100	0.879	0.957
Expected heterozygosity (H_E)	0.011	0.404	0.707
Inbreeding (*F*)	-0.079	0.849	0.957
Mean pairwise D_C	**0.208**	**0.005**	**0.035**
Mean pairwise F_{ST}	**0.225**	**0.010**	**0.035**

q, false discovery rate adjusted *p*-value.

In simulations of the collection of 125 seedlings in three ways (five seedlings drawn at random from each of 25 families taken at random from all families; five seedlings taken at random from each of five families drawn at random from each of the five seedling populations; and 125 seedlings drawn at random from all seedlings), the mean number of alleles collected was not significantly different across 100 iterations (Supplementary Table S4). The two measures of heterozygosity, however, were significantly different, and were highest among the samples drawn at random from among all seedlings without regard to family or population (H_O = 0.468 and H_E = 0.658) and lowest in the five seedlings from five random families from each of the populations sampling approach (H_O = 0.460 and H_E = 0.646). The sampling approach of selecting five seedlings from 25 random families was intermediate (H_O = 0.466 and H_E = 0.650). At the same time, inbreeding was the highest for the samples drawn at random without regard to family or population (F = 0.285) and lowest for the 25 families sampling approach (F = 0.274). Although these statistical tests found significant differences in the means of the genetic metrics, we note that these differences may not be biologically meaningful.

Discussion

Our range-wide genetic assessment of Table Mountain pine using microsatellites shows that this Appalachian endemic conifer consists of populations that exhibit relatively low differentiation among each other (F_{ST} = 0.039, Table 2). That differentiation was significantly but weakly associated with geographic distance among populations. We also found that southern seed zones were slightly but significantly more genetically differentiated than northern populations. Simulations of different sampling approaches for establishing conservation plantings underscored that including more families resulted in higher levels of allelic diversity and that reducing the number of families (from as many as ten to five) negatively affected the number of rare but not common alleles represented. Structuring a seedling sample by family or by family within population did not decrease allelic richness compared to a completely random selection of seedlings. These findings are all relevant to the *ex situ* genetic conservation of Table Mountain pine.

Patterns of Genetic Variation

Most of the results of this study point to an overall lack of genetic structure and geographic differences in genetic variation within Table Mountain pine, consistent with extensive gene exchange among its populations. At the same time,

Table 6. Mean number of alleles, percent of total common (≥5% percent) and rare (<5% frequency) alleles represented, observed heterozygosity (H_O), expected heterozygosity (H_E), and inbreeding fixation index (F) across 100 simulated seedling collections of five seedlings in five to nine or ten families in three populations of Table Mountain pine. Significantly different at p-values ≤ 0.05 from a multiple-sample Kruskal-Wallis test of group differences of sample draws within a population are in bold.

		Alleles													
				% of total			H_O			H_E			F		
	N	Mean	SD	All	Common	Rare	Mean	SD	% difference	Mean	SD	% difference	Mean	SD	% difference
Briery Branch															
10 families	50	**43.5**	1.7	83.6	100.0	52.7	0.479	0.02	-0.4	**0.661**	0.01	-1.4	**0.276**	0.03	-5.0
9 families	45	**43.0**	1.6	82.7	100.0	50.1	0.479	0.02	-0.4	**0.656**	0.01	-2.1	**0.276**	0.03	-5.2
8 families	40	**42.0**	1.8	80.8	99.8	45.1	0.481	0.03	-0.1	**0.652**	0.02	-2.6	**0.271**	0.05	-7.0
7 families	35	**41.4**	1.9	79.5	99.4	41.9	0.480	0.02	-0.1	**0.652**	0.02	-2.7	**0.265**	0.05	-8.9
6 families	30	**40.6**	1.7	78.1	98.9	38.9	0.485	0.03	0.8	**0.648**	0.02	-3.3	**0.252**	0.06	-13.5
5 families	25	**38.7**	1.8	74.4	97.5	30.8	0.475	0.03	-1.2	**0.639**	0.03	-4.6	**0.254**	0.07	-12.7
Greene Mountain															
9 families	45	**38.0**	1.2	90.5	97.9	66.8	0.469	0.02	-2.5	**0.580**	0.01	-2.9	**0.183**	0.03	-8.3
8 families	40	**37.7**	1.3	89.7	97.7	64.1	0.472	0.02	-1.9	**0.581**	0.02	-2.7	**0.180**	0.03	-9.7
7 families	35	**37.0**	1.5	88.0	97.3	58.1	0.470	0.02	-2.4	**0.579**	0.02	-3.0	**0.181**	0.04	-8.9
6 families	30	**36.5**	1.7	86.8	96.1	57.0	0.474	0.02	-1.5	**0.578**	0.02	-3.2	**0.175**	0.04	-12.2
5 families	25	**35.0**	1.7	83.4	94.8	46.8	0.473	0.03	-1.6	**0.573**	0.02	-4.1	**0.166**	0.05	-16.8
Walnut Fork															
9 families	45	**38.1**	1.2	93.0	100.0	71.2	0.467	0.02	3.2	**0.627**	0.01	1.2	**0.222**	0.03	-7.1
8 families	40	**37.8**	1.2	92.1	100.0	67.7	0.468	0.03	3.4	**0.624**	0.02	0.9	**0.218**	0.04	-8.9
7 families	35	**37.0**	1.4	90.1	99.6	60.7	0.468	0.03	3.3	**0.620**	0.02	0.1	**0.212**	0.04	-11.1
6 families	30	**35.9**	1.4	87.4	98.8	52.2	0.466	0.03	2.8	**0.615**	0.02	-0.6	**0.213**	0.05	-10.9
5 families	25	**35.2**	1.6	85.8	97.5	49.4	0.467	0.04	3.1	**0.610**	0.02	-1.4	**0.202**	0.05	-15.5

the study reveals some results may be relevant to the genetic conservation of Table Mountain pine. First, minor genetic differences appear to exist between northern and southern seed collection zones established for the species based on a cluster analysis of climate similarity (Jetton et al. 2015). Most importantly, populations in the southern seed zones (1/2) were on average more genetically distant and differentiated than those of the northern seed zones (3/4). Additionally, the northern populations had slightly higher allelic richness, although this difference was not statistically significant when accounting for multiple comparisons. It is unclear whether these differences among regions are meaningful enough to be reflected in gene conservation planning. These results seem to contradict previous allozyme findings that the populations with highest levels of diversity were at the northern and southern ends of the Table Mountain pine distribution (Gibson and Hamrick 1991). Second, we found relationships between population isolation and both differentiation and genetic diversity. Specifically, we detected (1) lower measures of heterozygosity for more isolated populations (>41 km to nearest neighbor) and (2) Mantel test correlations between interpopulation pairwise geographic distances and both pairwise genetic differentiation and genetic distance. These results are not surprising given the higher probability of successful interpopulation gene exchange via pollen dispersal that decreases from nearer to farther proximity (Ellstrand 1992). They indicate the potential utility of focusing gene conservation actions, such as genetic augmentation, on more isolated Table Mountain pine populations, because they are more susceptible to inbreeding and genetic drift (Jaramillo-Correa et al. 2009) and may have a reduced capacity to adapt to environmental change (Willi et al. 2006).

One objective of the study was to assess the utility of the Table Mountain pine seed zones that were established based on climatic data (Jetton et al. 2015) over a relatively short timeframe to guide seed collection efforts in the absence of phenotypic data from a common garden experiment. The genetic differences between the northern and southern seed zones were not strong, apparently because of extensive genetic admixture among populations, indicating that these seed zones are not a particularly useful tool for guiding future gene conservation efforts. Given the existence of regional within-species variation in important traits, such as cone serotiny (Brose and Waldrop 2010; Brose 2017), additional research using common garden studies is needed to better guide seed transfer decisions in Table Mountain pine.

We expected that Table Mountain pine would exhibit a pattern of interpopulation genetic structure similar to Carolina hemlock, an Appalachian endemic with limited long-distance pollen dispersal, because Table Mountain pine's wind-dispersed pollen is relatively large compared to other pine species (Della-Bianca 1990). The results of this study, however, indicate that it has levels of diversity and differentiation that are more similar to Fraser fir. Previous microsatellite analyses have shown that Fraser fir exists as a set of low-diversity and moderately inbred populations that are not much differentiated from each other (Potter et al. 2008) whereas Carolina hemlock populations are characterized by high inbreeding, low diversity, and extremely high differentiation (Potter et al. 2017). Our finding of relatively low among-population differentiation among Table Mountain pine populations is perhaps not surprising given this is the standard for conifer species with wind-dispersed pollen and seed (Hamrick et al. 1992; Hamrick and Godt 1996). Although Table Mountain pine has pollen that is relatively heavy for a pine, it is saccate (containing air-filled bladders thought to aid wind dispersal [Schwendemann et al. 2007; Grega et al. 2013]) as with other pines, which may allow for better interpopulation gene flow. Its seeds are also somewhat lighter than those of other yellow pines in the Appalachians (Krugman and Jenkinson 2008). Only a small amount of gene flow by either pollen or seed dispersal may be enough to avert genetic drift and inbreeding in isolated plant populations (Ellstrand 1992). This is in comparison with Fraser fir, which has seeds among the lightest in the *Abies* genus (Edwards 2008) and pollen that can be effectively dispersed across long distances, similar to the closely related balsam fir (*Abies balsamea* [L.] Mill.) (Janssen 1966). Hemlock pollen grains, on the other hand, lack sacci (Kurmann 1990) and are particularly susceptible to desiccation (Nienstaedt and Kriebel 1955). Combined with the small size and widely dispersed nature of the Carolina hemlock populations, this may make long-distance interpopulation gene flow a rare event in Carolina hemlock and apparently below the threshold needed to avoid genetic drift and high inbreeding (Potter et al. 2017). Many Carolina hemlock stands additionally are small and may be either mixed or pure (Jetton et al. 2008), whereas higher-elevation Fraser fir and Table Mountain pine populations are mostly or entirely composed of the species (Busing et al. 1993; Jetton et al 2015), with some Fraser fir populations encompassing a relatively large area compared with the other two species (Dull et al. 1988). This may help Table Mountain pine and Fraser fir avoid the low within-population genetic variation, genetic drift, and inbreeding associated with small population size (Young et al. 1996).

Conservation Sampling Simulations

An additional objective of this study was to assess the genetic implications of introducing flexibility in Camcore's conservation planting guidelines, which aim to deploy a minimum of ten open-pollinated families of five seedlings from each sampled population (Dvorak 2012). Sometimes the lack of seed availability for collection and the inadequate germination and survival of collected seeds make it impossible to meet these guidelines. Also, resources are limited for the establishment and maintenance of a long-term conservation planting, including land, nursery space, personnel, and materials. It is important, therefore, to avoid both oversampling to allow resources to be allocated to other uses and undersampling so that results are not ambiguous (Hoban et al. 2013). We therefore conducted two sets of simulations, using data from seedlings grown from Table Mountain pine seed collections, to assess the potential effectiveness of different sampling approaches in representing genetic variation in potential *ex situ* conservation plantings. In one set, we simulated how well the random selection of between five and nine or ten five-seedling open-pollinated families would represent genetic variation within three populations; in the second, we assessed the effectiveness of a sampling strategy based on both open-pollinated family and population structure versus one based only on family structure.

For our first set of simulations, we aimed to grow at least five seedlings from ten open-pollinated families representing each of five Table Mountain pine populations. The lack of adequate germination resulted in only one population (Briery Branch) having ten such families, whereas two others

(Greene Mountain and Walnut Fork) had nine. Using data from this real-life example of partial seedling germination success, we tested the genetic representativeness of simulated conservation plantings established from the seedlings of these three populations. Sampling the maximum number of families in each population on average captured between 83% and 93% of alleles with a decline of approximately 1.8% alleles with the removal of a family from the sample. Differences among populations likely stem from differences in the allelic richness of the seedling population from which the samples were drawn. For example, Briery Branch encompassed 52 alleles, compared to 42 and 41 for Greene Mountain and Walnut Fork, respectively, so an equal amount of sampling effort was less likely to represent as large a proportion of the total alleles in that population, particularly since it had more rare alleles (18 compared to ten each for the other two populations). A more complete sampling of populations known to have greater allelic richness may therefore require including a larger number of individuals or families. These results indicate that reducing the number of families included in a conservation sampling would not substantially affect the conservation of common alleles but would have a minor effect on the conservation of rare alleles and overall allelic richness represented by the conservation planting. In other words, conservation plantings that do not meet the ten-family target should still maintain well-adapted genetic material for future deployment, at least in terms of how they represent genetic variation expressed by putatively neutral molecular markers, but may not include some rare, adaptively advantageous alleles. Table Mountain pine may allow for additional flexibility in meeting Camcore's conservation planting guidelines given the strong evidence of extensive gene flow among its populations, although it would be advisable to include the full complement of ten five-seedling families for more isolated populations when possible, as well as those known to have higher allelic richness, such as Cherokee Orchard (#4), Briery Branch (#26), and Bull Run (#32).

Our other set of simulations did not show a significant difference in the mean number of alleles sampled when doing a completely random draw of 125 seedlings, sampling 25 five-seedling families at random, or sampling five seedlings from five families from each of the five populations. Heterozygosities are lower for the last of these sampling strategies, so choosing open-pollinated families at random from across populations, rather than sampling them equally from each of the populations present, may result in representing somewhat greater genetic variation by this measure. At the same time, this may be offset by the need to adequately conserve adaptive variation best represented at the family level and to generate balanced conservation plantings that can be the foundation of future tree breeding efforts. The range-wide genetic diversity results for Table Mountain pine indicate that this should include at least some families from isolated populations and from across the distribution of the species (especially from both the north and south) to ensure appropriate representation of genetic variation.

We acknowledge that the relatively small seedling sample sizes in the study were not ideal for the simulation studies; however, as noted above, we were limited by the real-world problem of inadequate seedling germination. Future studies along these lines should plan for this possibility by including additional open-pollinated families in the seed collection phase and by planting several extra seeds for each family included in the study. We also note that these results are relevant for species such as pines and firs, which have extensive interpopulation genetic exchange. Such a study conducted on a species like Carolina hemlock with limited interpopulation gene flow may have yielded different results.

Conclusions

Table Mountain pine is a relatively rare conifer that exists in a fragmented distribution across the central and southern Appalachian region, where it has declined in recent decades in the absence of the fire which plays an important role in its regeneration (Brose 2017; Lafon et al. 2021). Our range-wide analyses of 26 Table Mountain pine populations using seven highly polymorphic microsatellite SSR loci found that the species was inbred with relatively low differentiation among populations associated with geographic distance. Genetic differences between populations in the northern and southern seed zones were not strong, most likely the result of prolific interpopulation gene flow, indicating that these seed zones are not particularly useful for guiding future gene conservation efforts. Simulations of different sample approaches for establishing conservation plantings showed that plantings that do not meet the ten-family target still maintain well-adapted genetic material for future deployment, at least in terms of how they represent genetic variation expressed by putatively neutral molecular markers but may not include some rare, adaptively advantageous alleles. Choosing open-pollinated families at random from across populations, rather than sampling them equally from each population present, did not result in differences in alleles sampled but did represent greater heterozygosity. These findings indicate that it may be possible to meet the goals of conservation plantings by including fewer families per population or including families without regard to population.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary Materials

Supplementary data are available at *Forest Science* online.

Supplementary Table S1. Analysis of molecular variance (AMOVAs) of microsatellite data for Table Mountain pine; all variance components are significant at $p < 0.001$.

Supplementary Table S2. Description of the seven nuclear microsatellite loci used in the study, with size range, measures of genetic variation, inbreeding, deviation from Hardy-Weinberg equilibrium, and estimated null allele frequency for each, using data from the 498 seedlings grown from seed collected from five natural stands.

Supplementary Table S3. Measures of genetic variation for each of five seedling populations of Table Mountain pine, based on seven nuclear microsatellite loci.

Supplementary Table S4. Mean number of alleles, percent of total common (≥5%) and rare (<5% frequency) alleles represented, observed heterozygosity (H_O), expected heterozygosity (H_E), and inbreeding fixation index (F) across 100 simulated seedling collections in three collection methods, with bold differences among the three methods significantly different at p-values ≤ 0.05 from a multiple-sample Kruskal-Wallis test of group differences.

Supplementary Figure S1. Delta K (Evanno et al. 2005) results for Table Mountain pine STRUCTURE analysis, calculated using STRUCTURE HARVESTER (Earl and Vonholdt 2012).

Supplementary Figure S2. Bayesian information criterion (BIC) value versus number of genetic clusters in Table Mountain pine using adegenet.

Supplementary Figure S3. Association between individuals and each of the adegenet clusters in Table Mountain pine.

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