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ABSTRACT.—Shortleaf pine (*Pinus echinata*), Missouri's only native pine species, experienced intense logging during the post-Civil War Ozark lumber boom. Although recent restoration efforts have occurred primarily on federal and state land, efforts are being made to incorporate shortleaf management strategies on private land through the distribution of 1 y old bare-root seedlings grown from seeds at the George O. White nursery in Licking, Mo. In this study we examined the genetic structure of Missouri's historic shortleaf pines to determine if geographic compartmentalization of seed stock was necessary. Using twelve polymorphic microsatellite loci, we genotyped historic shortleaf pine wood core samples from eight counties in the Missouri Ozarks and needles from seedlings provided by the George O. White nursery. Missouri's historic shortleaf pine populations exhibited high heterozygosity and no observable pattern of genetic structure between individuals of varying geographic location, diameter at breast height (DBH), or altitude. However, private alleles found in all but one of the historic populations suggest the presence of a form of genetic diversity in Missouri's shortleaf pine populations that is not present in the George O. White nursery stock. To maintain high levels of genetic diversity over the long term and prevent overrepresentation of alleles from nursery stock in reintroduced shortleaf populations, managers should consider increasing the number of individuals used as seed sources for seedling propagation.

INTRODUCTION

Shortleaf pine (*Pinus echinata*), Missouri's only native pine species, has a long history of economic and ecological value to the state. It is estimated that shortleaf pine occurred across 2.7 million ha (Fletcher and McDermott, 1957), both on the sandy flint-ridges and the flat

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clay uplands of the Ozark Mountains (Liming, 1946; Cunningham, 2007). While pure stands did occur, shortleaf pine was commonly intermixed with hardwoods in pine-oak or oak-hickory stands.

Although the Ozark Mountains remained relatively untouched by early settlers due to their infertile rocky soils (Hammar, 1935), the post-Civil War era saw a large increase of settlers into the region looking to exploit the rich timber resources. From the late 1880s–1920s, the Ozarks were the center of Missouri's timber boom with an estimated yearly production of 724 million board feet at its peak in 1899 (Cunningham and Hauser, 1989). Shortleaf pine timber was extensively used in both general and railroad construction, leading to the rapid depletion of the resource by largely unregulated harvesting practices. The massive amount of logging, combined with unmanaged fire regimes, had devastating effects for Missouri's pine resource, reducing the population to the point where reseeding was severely inhibited (Guyette *et al.*, 2007). The decline of shortleaf pine populations allowed them to be replaced by oak-hickory and other hardwood stands, further impeding the growth of shade-intolerant pine seedlings and saplings and preventing pine restoration. Cunningham and Hauser (1989) estimated that shortleaf pine now covers only about 15% of its historic (*i.e.*, presettlement) range, or about 162,000 ha, in pure and mixed stands.

Today across the southeastern United States, shortleaf pine remains a significant commercial conifer species (Gagnon and Johnson, 2009) and serves as an important habitat and food source for wildlife at all successional stages (Masters, 2007). This disturbance-tolerant species has a low energy demand and an extensive root system compared to most competing species, making it extremely well adapted to the rocky and relatively infertile soils of the Ozarks. Once dominant in Ozark ecosystems, shortleaf pine stands were replaced after logging by scarlet and black oak, which have been declining since the 1980s. Guyette *et al.* (2007) found these declines tend to be followed by replacement by white oak rather than shortleaf pine. The combination of widespread oak decline and recent fire suppression, which closes canopy gaps and causes a buildup of leaf litter that covers the exposed soils necessary for pine germination, has made restoration efforts for shortleaf pine especially necessary since natural recruitment will likely remain low (Guyette *et al.*, 2007). Although management efforts have occurred primarily on federal and state land, efforts are being made to incorporate shortleaf management strategies on private land (Anderson *et al.*, 2016).

Operated today by the Missouri Department of Conservation (MDC), the George O. White (GOW) nursery in Licking, Mo., serves as the primary source of shortleaf pine seeds and 1 y old bare-root seedlings for public and private landowners across Missouri. The nursery was established by the U. S. Forest Service in 1935 to serve as a source of shortleaf pine seedlings for restoration efforts at Clark and Mark Twain National Forests (Gwaze *et al.*, 2007). Between 1935 and 1981, seeds for GOW were collected locally or purchased from orchards in neighboring states, especially from Arkansas (Gwaze *et al.*, 2007). After 1981 seeds were produced at Mt. Ida Seed Orchard (U.S. Forest Service) in Arkansas, where scions from 50 morphologically superior individuals from throughout the range in Missouri and within the Mark Twain National Forest, Mo., were grafted and established as seed orchard clones. After some trees at Mt. Ida were damaged or killed by a 1999 ice storm, the orchard's Mark Twain source population was reduced to 38 clones (Studyvin and Gwaze, 2007). Today, seed collected in 1984 and 2004 are the main sources used for plantings at GOW that produce seedlings for the MDC, the Mark Twain National Forest and the public (D. Biram, Missouri Department of Conservation, pers. comm.).

TABLE 1.—Coordinates for the eight counties were shortleaf pine wood samples were collected

Study Site	Sample ID	Latitude	Longitude
Barry Co.	B76	36.66842898	-93.64875603
Carter Co.	C21	37.05748432	-91.21629478
Dent Co.	D26	37.44678400	-91.64109911
Howell Co.	H1	36.83131509	-92.07601435
Ripley Co.	R10	36.71991759	-91.00791288
Shannon Co.	S19	37.02026507	-91.30121914
Texas Co.	T1	37.58622609	-92.02106721
Washington Co.	W51	37.99285065	-90.92960578

In general genetic studies of shortleaf pines have found high diversity within populations and a lack of population structure, suggesting substantial long-distance dispersal (Edwards and Hamrick, 1995; Raja *et al.*, 1997; Xu *et al.*, 2008; Stewart *et al.*, 2010). However, analyses of paternally inherited chloroplast microsatellites in pollen revealed significant genetic differentiation among populations at the local scale in southern Missouri (ranging in size from 750 to 900 ha; Dyer and Sork, 2001), suggesting that pollen dispersal is relatively restricted. The authors found a pattern of isolation by distance both within and between regions and suggest that at the local level the presence of dense vegetative structure influences pollen movement during reproductive events while at the regional level isolation by distance is the dominant influence. At a broader scale, Stewart *et al.* (2012) found that genetic differentiation among areas had increased in modern stands, likely due to the effects of habitat fragmentation, selection under increasing drought frequencies, and fire suppression. To better understand the dynamics of dispersal and inform management practices, it is increasingly important to document genetic diversity at local levels (Stewart *et al.*, 2016).

In this study we examined the genetic structure of historic shortleaf pine populations in the Missouri Ozarks. Knowledge of the genetic diversity and population structure of Missouri's shortleaf pine populations will help determine if seed stock used for restoration efforts should be compartmentalized based on geographic area. If genetic diversity is distributed geographically, then seed selection plans may be developed to protect unique stands. We predicted that relatively high genetic diversity exists across the total population with little differentiation or overall population structure between geographic locations, similar to the results of previous studies of shortleaf pine genetic diversity and population structure (Raja *et al.*, 1997; Koppelman *et al.*, 2007). By utilizing samples from multiple populations across the state and examining the diversity of George O. White nursery seedlings, we sought to provide more accurate management recommendations regarding shortleaf restoration within Missouri. This study is part of a statewide effort to restore Missouri's only native pine species, which once dominated the Ozark Mountains.

METHODS

SAMPLE COLLECTION

We collected shortleaf pine wood core samples (phloem, cambium and sapwood) from eight counties (Barry, Carter, Dent, Howell, Ripley, Shannon, Texas and Washington; *see* Table 1) in the Ozark Mountain region of southern and central-eastern Missouri between November 2005 and April 2007 (Fig. 1). Using Forest Inventory Analysis (FIA) data and aerial photographs from the Missouri Department of Conservation, we identified stands with a high abundance of

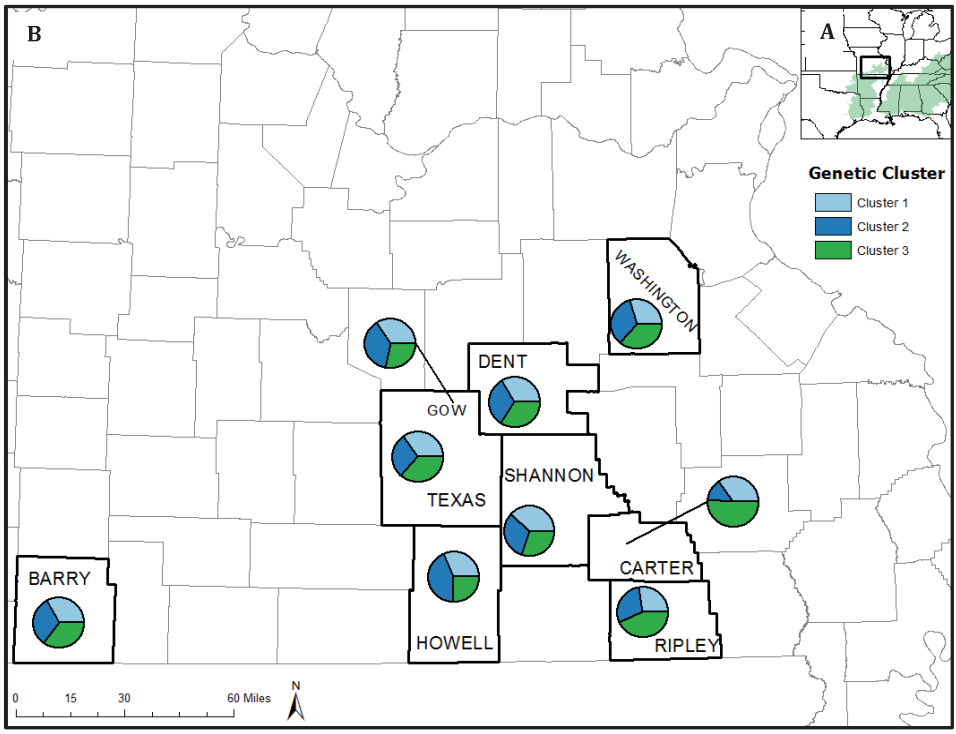

FIG. 1.—Geographic distribution of (**A**) shortleaf pines in the Midwestern United States and (**B**) distribution of the three genetic clusters across sampling locations for shortleaf pines in Missouri, U.S.A.

shortleaf pine and a mean diameter at breast height (DBH) of ≥38cm. A previous study of the correlation between shortleaf pine age and DBH shows that DBH may serve as a strong indicator of age in trees less than 100 y old (Guyette *et al.*, 2007); therefore, we used DBH as an indicator for tree age when selecting trees that had a high probability of representing remnants of historic (*i.e.*, presettlement) rather than restored stock for analysis. From the identified stands, geographically intermediate locations were selected for sampling to reduce bias due to gene flow and to represent the statewide range of shortleaf pine. We randomly selected sites at each location, and sampled trees located a minimum of 100 m apart.

To represent the seed stock used for restoration efforts, pine needle samples were clipped from 24 shortleaf seedlings at George O. White nursery in March of 2016. These included stands planted in the 1950s/1960s with presumed local seed (n = 9), in 1980 and 1984 with seeds produced from the Mt. Ida seed orchard clones (n = 10), and in 2004 using seeds from a large seed collection effort at Mt. Ida (n = 5; D. Biram, Missouri Department of Conservation, pers. comm.).

DNA EXTRACTION

Samples were stored at –80C until extraction in 2015 to preserve DNA quality. George O. White needle samples were extracted immediately after harvest. Using liquid nitrogen, we ground samples into a fine powder to break down plant cell walls and used the Qiagen

TABLE 2.—Microsatellite loci used for genotyping shortleaf pine in Missouri, U.S.A., including optimal annealing temperature (T_A), the fluorescent label added to the forward primer, average expected (H_E) and observed (H_O) heterozygosity values, number of alleles observed (A), and allele size range

Locus	T_A (C)	Label	H_E	H_O	A	Allele range (bp)
PtTX3034	56	NED	0.818	0.720	10	187–209
PtTX4058*	59	6-FAM	0.796	0.639	16	117–153
ript0031	58	VIC	0.855	0.883	15	241–277
ript0079	54	6-FAM	0.810	0.750	15	136–175
ript0126	59	VIC	0.862	0.751	18	165–207
ript0171*	56	PET	0.751	0.657	13	196–222
ript0367*	59	6-FAM	0.888	0.853	19	186–226
ript0619	50	PET	0.763	0.691	11	181–209
ript0629	59	NED	0.823	0.767	11	142–166
ript0852*	59	PET	0.355	0.167	4	199–205
ript0984*	58	6-FAM	0.725	0.501	12	213–239
RPtest9	59	VIC	0.723	0.584	7	266–296
Average			0.764	0.664	12.6	
StDev			0.139	0.189	4.4	

* Locus deviates from expected heterozygosity value under HWE in one or more populations

DNeasy Plant Mini Kit (Qiagen, Valencia, CA) to extract DNA following a modified protocol from Asif and Cannon (2005). DNA concentration and purity (A260/A280 reading) was measured in a Nano-drop ND1000 spectrophotometer (Thermo Scientific, Waltham, MA). We found some samples could not be reliably genotyped, therefore we selected 10 of the highest DNA-quality historic samples from each county for a total sample size of n = 80 and analyzed them along with the 24 samples from the George O. White nursery.

MICROSATELLITE GENOTYPING

We genotyped each sample at 12 polymorphic microsatellite loci developed and optimized by Nelson *et al.* (2007) for shortleaf and loblolly pine (Table 2). The polymerase chain reaction (PCR) was performed in 10 µl volumes containing 1X PCR Gold buffer (50 mM KCl, 10 mM Tris-HCL, Applied Biosystems, Foster City, CA), 1.5 mM BSA, 0.5 µM of fluorescently labeled forward (Table 2) and unlabeled reverse primers, 2 mM $MgCl_2$, 0.1 U AmpliTaq Gold polymerase (Applied Biosystems), and ~15 ng DNA. Single-locus amplifications were performed in an Eppendorf ep thermal cycler (Eppendorf, Hauppauge, NY) using a profile of denaturation at 95C for 10 min followed by 35 cycles of denaturation at 95C for 10 min, primer annealing at the optimized temperature (Table 2) for 1 min, primer extension at 72C for 1 min, and a final extension cycle at 72C for 10 min. All amplification reactions included a negative (no DNA) control to detect reagent contamination and a positive control to standardize allele scoring. Amplified fragments were separated in an ABI 3730 DNA Analyzer (Applied Biosystems) at the University of Missouri DNA Core Facility and scored against internal lane standards (Genescan LIZ 600) in GENEMARKER v 1.97 (SoftGenetics LLC, State College, PA).

POPULATION GENETIC ANALYSES

We used ARLEQUIN v3.5.1.2 (Excoffier and Lischer, 2010) to estimate the mean number of alleles (A), expected (H_E) and observed (H_O) heterozygosity values, and coefficient of

TABLE 3.—Population genetic results for shortleaf pine genotypes in Missouri, U.S.A., including average number of alleles (A) for each population, allelic richness (A_R), average observed (H_O) and expected (H_E) heterozygosity values, and inbreeding coefficients (F_{IS})

Population	County	N	A	A_R	H_O	H_E	F_{IS}
Western							
	Barry	10	6.33	6.33	0.596	0.789	0.140
	Howell	10	6.75	6.75	0.672	0.728	0.064
Eastern							
	Texas	10	6.75	6.75	0.683	0.776	0.120
	Dent	10	6.25	6.25	0.686	0.766	0.087
	Shannon	10	6.25	6.25	0.680	0.718	0.040
	Carter	10	6.42	6.42	0.690	0.778	0.023
	Ripley	10	7.17	7.17	0.702	0.798	0.117
	Washington	10	7.00	7.00	0.732	0.766	0.039
Average	Historic		6.62	6.62	0.680	0.765	0.079
StDev	Historic		0.35	0.35	0.039	0.028	0.044
Nursery							
	GOW	24	8.67	4.99	0.531	0.759	0.286

inbreeding (F_{IS}) for all geographic locations. Because the number of samples analyzed from George O. White was greater than the number analyzed from each of the historic sites, we estimated the number of alleles corrected for unequal sample sizes (allelic richness, A_R) in HP-RARE (Kalinowski 2005).

Tests for deviations from expected genotype frequencies under Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) were run using GENEPOP v1.2 (Raymond and Rousset, 1995). We assessed the significance of deviations after applying a Bonferroni correction to account for multiple testing. We used BOTTLENECK (Cornuet and Luikart, 1997) to test for the effects of a genetic bottleneck in both historic and George O. White samples using the stepwise mutation model (SMM), the infinite allele model (IAM), and the two-phase model (TPM, with 70% SMM). Results were tested for significance using a sign test and a Wilcoxon test in BOTTLENECK.

We analyzed population structure using pairwise fixation index (F_{ST}) values computed in ARLEQUIN and assessed their significance with permutation tests. We tested for isolation by distance by comparing matrices of pairwise genetic distances (F_{ST}) and geographic distances (km) between midpoints of counties in which samples were collected in IBDWS (Jensen *et al.*, 2005). In addition we analyzed the data using the Bayesian clustering program STRUCTURE v 2.3 (Pritchard *et al.*, 2000). We performed 10 replicates of each hypothesized number of independent genetic clusters (K) from one to nine. Burnin was set at 10^3 iterations, followed by 10^5 iterations. STRUCTURE HARVESTER (Earl and VonHoldt, 2012) was used to model and display these replicates, as well as to calculate the log probability of the data [LnP(K)] and the rate of change in the log probability between successive K values (ΔK, Evanno *et al.*, 2005).

RESULTS

HISTORIC POPULATIONS

The average number of alleles detected for each locus across all historic populations was 6.62 (range 6.25–7.17, Table 3). Expected (H_E) and observed (H_O) heterozygosity values

were relatively high for each county and average H_E and H_O values were 0.765 and 0.680, respectively, across all sampled counties. Barry County had the lowest observed heterozygosity value at 0.596, while Washington County had the highest value of 0.732.

Heterozygosity values deviated from expectations under HWE at five loci: locus PtTX4058 in Dent and Ripley counties, locus ript0171 in Ripley county, locus ript0367 in Howell and Washington counties, locus ript0852 in Carter county, and locus ript0984 in Texas county (Table 3). Linkage disequilibrium was not observed between any pairs of loci. Since the pattern of deviations from HWE were not consistent across loci within populations; all loci were retained for subsequent analyses.

In BOTTLENECK we did not detect the signature of a genetic bottleneck in any historic population using any of the mutation models. Inbreeding coefficients (F_{IS}) varied from a low of 0.039 in Washington County to a high of 0.117 in Ripley County (Table 3), indicating low to moderate levels of inbreeding in historic populations. Private alleles were observed in seven of the eight historic populations, with numbers ranging from one in Dent County to six in Carter County (Appendix Table 1).

Pairwise comparisons of F_{ST} between populations showed low levels of genetic differentiation in most instances (Appendix Table 2), and we found no evidence of isolation by distance ($Z = 66.25$, $r = 0.356$, P $r>0 = 0.791$, P $r<0 = 0.209$). However, Carter County was found to be significantly different from Dent, Howell and Texas Counties, and Barry County differed significantly from both Howell and Washington Counties (Appendix Table 2). Using STRUCTURE and STRUCTURE HARVESTER, we detected three ancestral genetic clusters across southeastern Missouri. However, the value of ΔK (11.13) was lower than that expected for significantly differentiated clusters. Moreover, the proportion of each of the three genetic clusters detected was roughly equal across all counties except Carter County, with each cluster accounting for about one-third of the individuals. Therefore, we conclude that there is no geographically based population structure across the range of shortleaf pine in Missouri. In addition we found no evidence of population structure between trees with different DBH or at different altitudes.

GEORGE O. WHITE NURSERY

The George O. White nursery pines exhibited lower overall genetic diversity than historic populations (Table 3). Although expected heterozygosity was very similar to that of the historic populations, observed heterozygosity was lower. The average number of alleles was higher, but when corrected for unequal sample size using rarefaction (allelic richness), the value of 4.99 alleles/locus was lower than the average of 6.62 alleles/locus observed in historic populations (Table 3). We detected seven private alleles at very low frequencies in five loci. We detected the signature of a genetic bottleneck using the stepwise mutation model (sign test $P = 0.004$, Wilcoxon one-tail test $P = 0.004$). At 0.286, the level of inbreeding (F_{IS}) detected in the George O. White samples was twice that detected in any of the historic populations (Table 3).

DISCUSSION

Our results are consistent with previous studies that have shown high within-population variation and little divergence among populations in long-lived woody tree species (Porth and El-Kassaby, 2014). The number of samples we examined from each of the historic populations is relatively small ($n = 10$) and does not allow us to make strong inferences about the levels of genetic diversity present within each of the historic populations. However,

our sample sizes are on par with those of previous studies that used from seven to 20 samples from six to 18 stands/populations (Edwards and Hamrick, 1995; Raja *et al.*, 1997; Dyer and Sork, 2001; Xu *et al.*, 2008; Stewart *et al.*, 2010, 2012) and found very similar results. It is difficult to compare our within-population variation based on microsatellite loci with that estimated using allozymes (Edwards and Hamrick, 1995; Raja *et al.*, 1997) or AFLPs (Xu *et al.*, 2008), and many of the recent studies of shortleaf pines provide data only for among population variability (Stewart *et al.*, 2010, 2012, 2016). In their evaluation of loblolly pine (*Pinus taeda*) microsatellite loci for use in shortleaf pines, Nelson *et al.* (2007) used six shortleaf pines from Mississippi, and their observed heterozygosity (0.768) did not differ significantly from that observed in our study ($t = 1.037$, $df = 22$, $P = 0.311$). Comparisons with other pine species studied using microsatellite loci reveal high allelic diversity (9.37 effective alleles/locus) and heterozygosity (0.847) in 21 native populations of Scots pines (*Pinus sylvestris* L; Belletti *et al.*, 2012) across Italy. In 17 populations of red pines (*Pinus resinosa*) in northeast North America, Boys *et al.* (2005) found a comparable level of allelic diversity but lower heterozygosity ($H_E = 0.508$, $H_O = 0.185$), consistent with a genetic bottleneck for red pine during glacial events (Jackson *et al.*, 2000).

We found shortleaf pine populations across southeastern Missouri exhibit relatively high historic genetic diversity despite dramatic declines in population sizes during and since the Ozark timber boom. No observable pattern of genetic structure was seen between individuals of varying geographic location, DBH, or altitude. Pairwise F_{ST} comparisons show little genetic differentiation among geographic locations, further supporting the lack of population structure inferred by our analyses in STRUCTURE. While Carter County was found to differ from three of the other seven counties, it also had a low inbreeding coefficient (Table 3). It had seven low frequency private alleles, five of which were at locus ript0367 (Appendix Table 1). Collectively, we interpret these data as evidence of genetic drift in a relatively isolated subpopulation. Overall, our data support the results of previous studies of this species (Edwards and Hamrick, 1995; Raja *et al.*, 1997; Xu *et al.*, 2008; Stewart *et al.*, 2010) that found high variability within sampled populations and little to no distinguishable population structure based on geography, DBH, or altitude.

The lack of population structure supports a pattern of long distance dispersal via pollen or seeds. In this study we detected low to moderate levels of inbreeding within each population, which supports the notion of restricted pollen movement at the local level. However, only a small number of migrants are needed in each generation to prevent the development of significant genetic structure at the regional level (Wright, 1931). In Scots pine (*Pinus sylvestris*), Robledo-Arnuncio (2011) demonstrated effective pollen flow (up to 6.7% male gamete migrants) over distances from 50–100 km. Shortleaf pines produce winged seeds that can be distributed as far as 90 m by wind (Lawson, 1990). Such long-distance pollen and seed dispersal could facilitate the low overall genetic differentiation observed between counties in Missouri in our study and in previous studies (Raja *et al.*, 1997).

Although we did not detect significant differentiation among most populations, the presence of private alleles in all but one historic population suggests that a low level of differentiation may exist. Unlike previous studies of shortleaf genetic structure (Raja *et al.*, 1997), no geographic structure of private allele presence was observed. Low-frequency alleles, which were not always present in the George O. White nursery stock, suggest the presence of genetic diversity at nuclear loci in Missouri's shortleaf populations that may be important to this species as it responds to environmental changes and should be preserved through management efforts.

Often, individuals at the edge of their species range exhibit lower than expected genetic diversity due to edge effects (Eckert and Samis, 2008). Although no significant signatures of population structure were detected, the population at the western edge of the statewide range exhibited the lowest heterozygosity and highest level of inbreeding. It is likely that the population in Barry County has experienced edge effects, which could reduce its overall fitness. Since Missouri accounts for the northwestern most portion of the nationwide distribution of shortleaf pine and considering the seed dispersal potential of the species, it is possible that the lower than expected heterozygosity values and higher inbreeding coefficients exhibited by all historic populations could be explained by edge effects.

In addition to individuals at the edge of a species distribution, small populations are also subject to the detrimental effects of inbreeding and reduced diversity such as reduced ability to adapt to changing local conditions (Shaffer, 1981; Caughley, 1994). We detected higher levels of inbreeding in George O. White saplings than any historic population, as well as the signature of a genetic bottleneck, although only under the stepwise mutation model. Given that all MDC-initiated restoration of shortleaf pine in Missouri originates from just two seed propagation events from the same individuals, it is possible that over time alleles from the George O. White stock may be overrepresented in restored populations. Despite this, it appears that managers at George O. White nursery have largely captured the levels of historic genetic diversity and allelic richness in nursery stock.

To maintain high levels of genetic diversity over the long term and prevent overrepresentation of alleles from nursery stock in Missouri's re-introduced shortleaf populations, managers could consider increasing the number of individuals used as seed sources for seedling propagation. Since no population structure was observed across southeastern Missouri and no significantly unique stands were identified, our findings suggest seed compartmentalization is not needed to proceed with replanting efforts across the state. The maintenance of historic genetic diversity is essential in the long-term management strategy of these ecologically and commercially valuable native trees and should remain a priority in future shortleaf re- establishment efforts.

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APPENDIX TABLE 1.— Allele frequencies by population for twelve microsatellite loci for eight historic shortleaf pine populations and the George O. White nursery (GOW). Private alleles are in bold

Locus	Allele/n	Barry	Carter	Dent	Howell	Ripley	Shannon	Texas	Washington	GOW
PtTX3034	N	10	10	10	10	10	10	10	10	24
	187	0.300	0.100	0.100	0.200	0.050	0.100	0.300	0.100	0.125
	193	-	-	-	-	**0.050**	-	-	-	-
	195	0.100	0.050	0.150	0.200	0.050	0.150	0.100	0.200	0.104
	197	-	0.050	-	-	0.050	-	-	0.050	-
	199	0.300	0.500	0.300	0.300	0.350	0.450	0.100	0.150	0.396
	201	0.050	0.150	-	0.150	0.050	0.150	0.150	0.100	0.125
	203	-	-	0.050	-	0.050	-	0.050	-	0.042
	205	0.150	0.100	0.250	0.100	0.250	0.050	0.150	0.250	0.104
	207	0.100	0.050	0.150	0.050	0.100	0.050	0.150	0.150	0.104
	209	-	-	-	-	-	**0.050**	-	-	-
PtTX4058	N	8	6	8	10	10	10	10	10	24
	117	-	-	-	**0.050**	-	-	-	-	-
	125	0.063	0.167	0.189	0.150	0.100	-	-	0.200	0.125
	127	0.375	0.417	0.313	0.500	0.253	0.524	0.358	0.644	0.274
	129	-	-	-	-	-	-	0.050	0.050	0.042
	131	-	0.083	0.125	-	0.100	0.050	0.100	0.050	0.042
	133	-	-	-	-	-	-	-	-	**0.021**
	135	-	-	0.063	-	-	0.050	0.100	-	-
	137	0.125	-	0.063	-	-	0.100	0.151	-	0.083
	139	0.063	-	-	-	0.100	0.050	-	-	0.042
	141	0.125	-	-	-	0.050	-	-	-	0.021
	143	**0.063**	-	-	-	-	-	-	-	-
	145	-	0.167	0.125	0.100	0.151	0.151	0.100	-	-
	147	0.189	0.167	0.125	0.151	0.201	0.100	0.151	0.050	0.253
	149	-	-	-	0.050	0.050	-	-	0.050	0.063
	151	-	-	-	-	-	-	-	-	**0.021**
	153	-	-	-	-	-	-	-	-	**0.021**
ript0031	N	10	7	10	10	10	10	9	10	24
	238	0.100	-	0.050	-	-	-	-	-	-
	241	0.250	-	0.150	0.150	0.050	0.050	-	0.200	-
	244	0.400	0.143	0.350	0.200	0.100	0.300	0.278	0.150	0.333
	247	0.100	0.214	0.250	0.150	0.250	0.300	0.167	0.050	0.125
	250	0.050	0.143	-	0.050	-	0.150	-	0.050	0.021
	253	0.050	0.143	-	0.150	0.050	-	-	0.150	0.021
	256	-	0.143	-	0.150	0.100	0.050	0.278	0.200	0.146
	259	-	0.071	0.100	0.050	0.250	-	-	0.050	0.062
	262	-	-	0.050	0.050	0.100	-	0.111	0.050	0.104
	265	0.050	0.143	0.050	0.050	-	0.050	0.167	0.050	0.104
	268	-	-	-	-	0.050	-	-	0.050	0.021
	271	-	-	-	-	-	0.050	-	-	0.021
	274	-	-	-	-	-	0.050	-	-	0.021
	277	-	-	-	-	-	-	-	-	**0.021**
	283	-	-	-	-	**0.050**	-	-	-	-
ript0079	N	10	10	10	10	10	10	10	10	23
	136	0.300	0.250	0.250	0.150	0.150	0.100	0.050	0.150	0.304
	139	0.300	0.150	0.450	0.450	0.350	0.500	0.450	0.350	0.283

APPENDIX TABLE 1.— Continued

Locus	Allele/n	Barry	Carter	Dent	Howell	Ripley	Shannon	Texas	Washington	GOW
	142	0.050	0.200	0.050	0.050	0.150	0.100	0.050-	-	0.043
	145	-	0.100	0.050	-	0.100	0.050	-	0.050	-
	148	-	-	0.050	-	-	-	0.050	-	0.022
	151	0.050	0.050	-	0.050	0.050	0.100	0.050	0.050	-
	154	0.100	0.050	-	-	-	-	0.050	0.050	-
	157	0.050	-	0.050	0.150	0.100	0.050	0.100	0.200	0.087
	160	-	0.050	-	0.050	-	-	0.100	0.100	0.130
	163	-	0.100	-	0.050	-	0.050	-	-	0.022
	166	-	-	0.050	-	0.050	-	-	-	-
	169	0.050	0.050	0.050	-	0.050	0.050	-	0.050	0.109
	172	-	-	-	-	-	-	**0.100**	-	-
	175	**0.100**	-	-	-	-	-	-	-	-
	178	**-**	-	-	**0.050**	-	-	-	-	-
ript0126	N	8	9	10	10	10	10	10	10	24
	165	-	-	-	-	0.050	-	-	0.050	0.042
	167	-	-	-	-	-	0.050	-	-	0.042
	169	-	-	**0.100**	-	-	-	-	-	-
	171	-	-	0.150	0.100	-	-	-	0.100	0.042
	173	0.125	0.056	-	0.050	-	0.050	-	-	0.021
	175	-	0.056	-	-	0.100	-	-	-	-
	177	0.063	0.056	0.100	0.050	0.050	0.100	0.050	0.050	-
	179	0.250	0.444	-	0.050	0.100	0.100	0.150	0.150	0.146
	181	0.125	0.389	0.300	0.350	0.100	0.250	0.350	0.300	0.208
	183	0.063	-	0.150	0.050	0.200	0.150	0.050	0.150	0.167
	185	0.063	-	0.200	0.050	0.200	0.100	0.200	-	0.083
	187	-	-	-	0.050	0.050	0.050	-	0.050	0.083
	191	-	-	-	-	-	-	-	-	**0.021**
	193	-	-	-	-	0.050	-	-	0.050	-
	201	-	-	-	0.100	-	-	0.100	-	-
	203	0.063	-	-	-	0.050	-	0.100	-	0.021
	205	0.125	-	-	0.100	-	0.050	-	0.050	0.042
	207	0.125	-	-	0.050	0.050	0.100	-	0.050	0.083
ript0171	N	9	10	10	9	10	10	10	10	24
	196	0.444	0.200	0.250	0.111	0.250	0.250	0.350	0.150	0.375
	200	-	-	0.050	-	0.050	-	-	-	-
	202	-	-	0.100	-	0.150	0.200	0.050	0.150	0.167
	204	-	-	-	-	-	-	-	-	**0.021**
	206	0.444	0.600	0.350	0.444	0.300	0.400	0.300	0.500	0.271
	208	0.056	0.050	0.100	-	0.100	-	-	-	0.042
	210	-	0.050	-	0.056	-	-	-	-	-
	212	0.056	0.050	0.050	0.167	-	0.100	0.150	0.050	0.083
	214	-	-	-	**0.056**	-	-	-	-	-
	216	-	-	-	0.111	0.050	-	0.100	0.050	0.042
	218	-	0.050	0.050	-	0.050	-	0.050	0.050	-
	220	-	-	0.050	0.056	**-**	0.050	-	-	-
	222	-	-	-	-	0.050	-	-	0.050	-
ript0367	N	9	8	9	9	9	9	10	10	21
	184	-	-	0.056	0.167	-	-	0.150	0.050	-

APPENDIX TABLE 1.— Continued

Locus	Allele/n	Barry	Carter	Dent	Howell	Ripley	Shannon	Texas	Washington	GOW
	186	0.167	-	0.333	0.167	-	-	0.150	0.150	0.262
	188	-	-	-	-	-	-	**0.050**	-	-
	190	0.056	-	-	-	-	-	0.100	-	0.048
	194	0.056	-	0.056	-	-	-	0.050	-	0.048
	200	0.111	-	-	0.056	0.056	-	-	-	0.024
	202	-	-	-	0.167	0.167	-	-	0.050	0.095
	204	-	-	-	-	-	-	-	-	**0.024**
	206	-	0.063	-	-	-	-	-	-	0.048
	208	0.111	0.188	0.167	0.056	0.056	0.111	0.050	0.050	-
	210	0.167	0.125	0.222	0.056	0.222	0.167	0.100	0.150	0.190
	212	-	0.063	0.111	0.056	0.222	0.167	0.150	0.100	0.095
	214	0.222	0.125	0.056	0.167	0.167	0.333	0.150	0.250	0.071
	216	0.111	0.125	-	0.111	0.111	0.222	0.050	0.200	0.095
	218	-	**0.062**	-	-	-	-	-	-	-
	220	-	**0.062**	-	-	-	-	-	-	-
	222	-	**0.062**	-	-	-	-	-	-	-
	224	-	**0.062**	-	-	-	-	-	-	-
	226	-	**0.062**	-	-	-	-	-	-	-
ript0619	N	10	10	10	10	10	10	10	10	24
	181	0.650	0.500	0.500	0.350	0.300	0.450	0.350	0.250	0.417
	185	0.100	-	0.150	0.200	0.300	0.100	0.050	0.100	0.125
	187	-	-	-	-	-	-	**0.050**	-	-
	195	-	-	-	0.050	-	-	0.100	0.100	0.042
	197	-	-	0.050	-	-	-	-	-	0.021
	199	-	-	0.100	0.050	-	0.100	-	-	0.042
	201	0.100	0.150	0.050	0.150	-	-	0.050	0.100	0.021
	203	0.050	0.200	0.100	0.150	0.250	0.200	0.100	0.150	0.208
	205	0.100	0.150	0.050	-	0.150	0.150	0.150	0.200	0.125
	207	-	-	-	-	-	-	0.050	0.050	-
	209	-	-	-	0.050	-	-	0.100	0.050	-
ript0629	N	9	10	10	10	10	10	10	9	24
	142	-	**0.100**	-	-	-	-	-	-	-
	146	0.111	0.300	-	-	-	0.050	0.050	-	-
	150	-	-	-	-	-	**0.050**	-	-	-
	152	-	-	0.050	-	-	0.100	-	-	-
	154	0.111	-	-	-	-	-	-	0.222	0.083
	156	-	0.050	-	-	-	-	0.050	-	0.083
	158	-	0.100	0.150	0.050	0.100	0.050	0.050	0.056	0.042
	160	0.222	0.050	0.200	0.200	0.200	0.200	0.250	0.333	0.188
	162	0.222	0.050	0.350	0.300	0.500	0.200	0.300	0.278	0.292
	164	0.167	0.150	0.150	0.200	0.150	0.200	0.200	0.111	0.167
	166	0.167	0.200	0.100	0.250	0.050	0.150	0.100	-	0.146
ript0852	N	5	9	10	10	9	10	10	10	24
	199	0.300	0.333	-	-	0.111	-	-	0.050	-
	201	0.300	0.556	0.800	0.850	0.778	0.950	0.900	0.900	0.833
	203	0.200	0.111	-	-	-	-	-	-	0.021
	205	0.200	-	0.200	0.150	0.111	0.050	0.100	0.050	0.146

APPENDIX TABLE 1.— Continued

Locus	Allele/n	Barry	Carter	Dent	Howell	Ripley	Shannon	Texas	Washington	GOW
ript0984	N	8	7	10	10	10	10	10	10	23
	213	-	-	0.050	0.150	0.050	0.100	0.150	0.150	0.174
	215	-	0.071	-	-	-	-	-	-	0.109
	217	-	-	0.100	-	-	-	-	-	0.065
	219	-	0.071	-	-	-	-	-	0.050	0.022
	221	0.062	-	0.200	0.100	0.050	0.100	0.050	0.100	0.087
	223	0.625	0.500	0.400	0.650	0.450	0.650	0.400	0.350	0.261
	225	0.125	-	0.050	-	0.050	-	0.100	-	0.043
	227	-	-	-	-	-	-	-	0.050	0.043
	231	0.125	0.143	0.100	-	0.250	0.050	0.150	0.100	-
	233	0.062	0.214	0.100	-	0.100	0.100	0.150	0.200	0.174
	237	-	-	-	0.050	0.050	-	-	-	0.022
	239	-	-	-	**0.050**	-	-	-	-	-
RPtest9	N	10	10	10	10	10	9	10	10	24
	266	-	0.050	-	-	0.050	-	0.050	0.100	-
	272	0.150	0.150	0.050	0.100	0.150	0.111	0.300	0.100	0.167
	281	0.200	0.050	-	0.050	0.150	0.111	-	0.100	0.042
	284	0.250	0.300	0.400	0.750	0.250	0.500	0.400	0.400	0.667
	287	0.150	0.200	-	0.050	0.150	0.111	0.150	-	0.062
	293	0.050	0.100	0.300	-	0.050	-	0.050	-	0.021
	296	0.200	0.150	0.250	0.050	0.200	0.167	0.050	0.300	0.042

APPENDIX TABLE 2.— Above diagonal: pairwise F_{ST} values between counties across the range of Shortleaf Pine in Missouri. Asterisks (*) denote statistically significant values. Below diagonal: pairwise distances (km) between midpoints of counties in which samples were collected

County	Barry	Carter	Dent	Howell	Ripley	Shannon	Texas	Washington
Barry	-	0.022	0.009	0.031*	0.016	0.020	0.018	0.028*
Carter	327	-	0.032*	0.030*	0.012	0.012	0.023*	0.019
Dent	294	122	-	0.006	0.004	0.003	0.001	0.007
Howell	261	108	140	-	0.022	0.007	-0.001	-0.001
Ripley	386	66	180	113	-	0.004	0.006	0.007
Shannon	302	53	69	93	117	-	-0.001	0.003
Texas	238	123	60	80	189	68	-	-0.001
Washington	380	130	113	219	214	141	158	-