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REVIEW

The genetics of shortleaf pine (*Pinus echinata* mill.) with implications for restoration and management

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Abstract Shortleaf pine (*Pinus echinata* Mill.) is an important commercial timber resource and forest ecosystem component in the southeastern USA. The species occurs in mainly drier sites as an early- to mid-successional species, is fire-adapted, and it plays an important role in the fire ecology of the region. However, shortleaf pine genetics are not well-studied, especially in this era of molecular genetics and genomics. Most genetics research about the species has focused on provenance testing. Generally, shortleaf pine performs well in colder areas, when compared to loblolly pine (*Pinus taeda* L.), a close relative, which is faster growing and the most common plantation species in the region. Though not as advanced in genetic improvement as loblolly pine, tree breeders have improved shortleaf pine in one to two generations of selection, and diverse, genetically improved shortleaf pine materials are available to foresters and landowners throughout the southeastern USA. Researchers have also studied the genetic variation of shortleaf pine using various molecular markers and have found that shortleaf pine is generally a prolific outcrosser, a trait it shares with other non-isolated members of the family Pinaceae. In recent years, however, it has shared less genetic material across long ranges, probably because of habitat fragmentation. Various anthropogenic factors also affect shortleaf pine's future, as recent studies show that shortleaf pine introgression with loblolly pine puts the species—and the resiliency of southeastern forests—at risk. Importantly, fire exclusion is a likely cause of the increase in introgression. Herein, we provide further details and up-to-date genetic information and resources for foresters and ecologists interested in the restoration and management of shortleaf pine.

Keywords Shortleaf pine · Genetics · Conservation · Silviculture

Introduction

Shortleaf pine (*Pinus echinata* Mill.) has the largest natural range of the pines in the southeastern USA and was once the dominant species across a large part of the region (Mohr and Roth 1897; Mattoon 1915). Fire exclusion and changes in land use and management have led to a reduction in the coverage and importance of shortleaf pine within stands where it occurs (Oswalt 2011). In addition, shortleaf pine is threatened by hybridization with loblolly pine (*Pinus taeda* L.) (Stewart et al. 2012). Therefore, a baseline understanding of current genetic information and resources will contribute to developing a plan to maintain and restore shortleaf pine and help identify topics that might require additional research.

While shortleaf pine is an extremely important tree species for forest ecosystems and the forest products industry of the southeastern USA, we have found little synthesis and review regarding the genetics of this species. The most comprehensive review of shortleaf pine genetics is included in a broader

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review of southern pines and is now 40 years old (Dorman 1976). In light of this absence of synthesis and the high interest in shortleaf pine as a foundational species for large-scale ecosystem restoration, we prepared this review paper. In our review, we first provide important ecological and silvicultural details to put the genetics information into perspective. Next, we include a detailed review of the genetics of shortleaf pine, including information on phylogeny, biogeography through provenance variation and genetic diversity studies, cytogenetics and genomics, genetics and tree improvement, and hybridization and introgression.

We found that most of the studies of shortleaf pine genetics focus on provenance testing, and we report the important works, as well as the overall themes and conclusions of the body of literature. The sources of seeds for shortleaf pine plantings and the nursery industry (public and private) are immensely important, but hitherto, little can be found in the literature about genetic resources for landowners and land managers, so we provide key details about current seed orchards and breeding programs. In recent years, researchers have investigated important issues surrounding shortleaf pine's propensity to hybridize and introgress with other pine species, especially loblolly pine. We provide an overview of historical and modern research into this phenomenon, which may threaten the genomic integrity of shortleaf pine.

Given that forests with a major shortleaf pine component decreased by nearly half since the 1980s—and probably by much more compared to the 1800s (Mohr and Roth 1897)—and that most of the remaining shortleaf pines are large-diameter trees (Oswalt 2011), professional land managers and government agencies need to be aware of the most current scientific information about the genetics of shortleaf pine in planning their ecosystem restoration programs. We hope that this review will provide land managers and forest researchers with good fundamental knowledge of shortleaf pine genetics and give direction to future research on this important forest tree species.

Ecological and silvicultural context

Shortleaf pine's current range extends to northern Florida (9 °C average minimum temperature) in the southeast, east Texas (5 °C average minimum temperature) in the southwest, southern Missouri (−7 °C average minimum temperature) in the northwest, and southern Pennsylvania (−8 °C average minimum temperature) in the northeast (Fig. 1). The species is largely excluded from the Mississippi River floodplain, which creates a large gap between the two halves of shortleaf pine's range.

Most of shortleaf pine's range is sympatric with loblolly pine, which has important implications to issues of hybridization between the two species. However, about a third of shortleaf pine's range is allopatric with respect to loblolly pine. West of the Mississippi River, that allopatric range includes the Ozark and Ouachita Mountains of Arkansas, Oklahoma, and Missouri, where it is the only naturally occurring pine species. East of the Mississippi River, that allopatric range includes the Cumberland Plateau in Tennessee and Kentucky, and the Appalachian-Blue Ridge Mountains from Alabama to Pennsylvania and New Jersey; here, shortleaf pine is rarely found in pure stands, and is commonly mixed with Virginia pine (*Pinus virginiana* Mill.), pitch pine (*Pinus rigida* Mill.), and Table Mountain pine (*Pinus pungens* Lamb.), as well as some hardwoods, especially oaks (spp.).

Shortleaf pine is an early successional species, though it can dominate certain landscapes over time, especially when disturbance from surface fire is common. On mesic sites, it does not compete well with loblolly pines or associated hardwoods; conversely, mature trees and dominant stands can often occur on upland or southern slopes (Lawson 1990). The species is well adapted to fire, as seedlings and saplings can re-sprout from a basal crook following topkill from fire (Mattoon 1915). Shortleaf pine is quite resistant to fusiform rust (caused by *Cronartium quercuum*), rarely being damaged by the disease (Powers et al. 1984). Economically, shortleaf pine is one of the most important softwood timber species in the southeastern USA after loblolly pine. Loblolly pine grows more vigorously than shortleaf pine on mesic or wetter soil, and fully grown trees are well adapted to fire. Woods-run loblolly pine trees tend to be susceptible to fusiform rust, but improved varieties are generally rust resistant. When choosing planting stock, foresters should consider the risk of planting loblolly pine from eastern sources such as North Carolina in areas prone to drought. Shortleaf pine is widely known to be more drought-tolerant than loblolly pine. When conducting a provenance test of loblolly pine on sites in Arkansas and Oklahoma, Lambeth et al. (1984) found that western loblolly pines grew more slowly than the eastern sources but exhibited improved survival during drought. They argued that to plant eastern pines in western locations, one needs to consider a risk and reward analysis. However, they only discussed potential issues in terms of profitability and did not consider ecological impact.

On a typical site, the site index and early volume growth for loblolly pine and slash pine (*Pinus elliottii* Engelm.) exceed that for shortleaf pine, which closely approximates the site index of longleaf pine (*Pinus palustris* Mill.) (Livingston 1972; Branan and Porterfield 1971). Among the other southern pines, shortleaf pine most often co-occurs with loblolly pine. In these mixed stands, shortleaf pine grows more slowly than loblolly pine and without disturbance, will be displaced by loblolly pine over the long run (Bragg and Shelton 2010). When comparing plantation growth of current stands, the advantage of loblolly pine over shortleaf pine has probably increased due to greater efforts and advances in loblolly pine

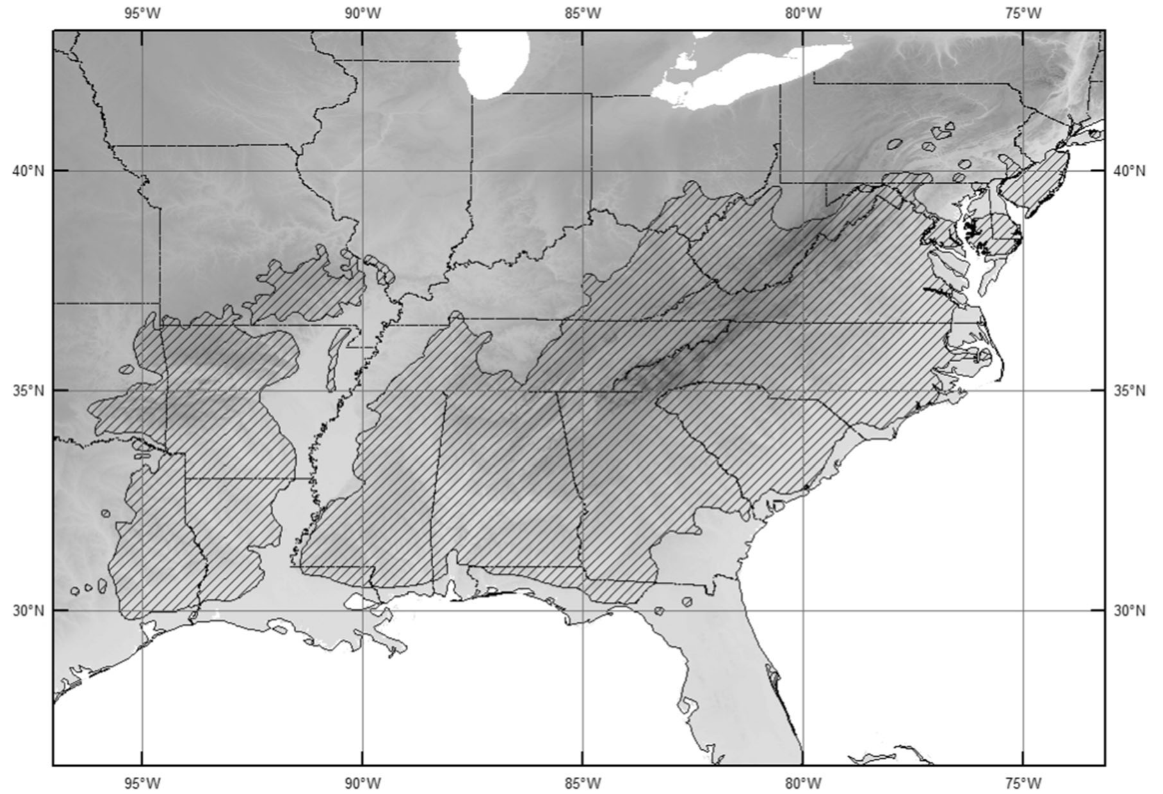

Fig. 1 Shortleaf pine occurs in many upland areas over a wide-range in southeastern USA (Critchfield and Little, 1966)

genetics through breeding. Most shortleaf pine seedlings available are grown from open-pollinated seeds from first-generation selections. In comparison, third-generation loblolly pine seedlings are commercially including seedlings from mass-controlled pollinated, full-sib families. A recent study that compared genetically improved shortleaf pine and loblolly pine seedlings available from the Western Gulf Tree Improvement Cooperative available at time of planting, which was in 2002, found that loblolly pine stands had 2.5 times the stem volume than did shortleaf pine stands after ten growing seasons, even though they were planted north of the natural range of loblolly pine in southeastern Oklahoma (Dipesh et al. 2015).

While genetically improved shortleaf pine materials are available, the species can respond well to natural regeneration management. Approaches including the seed-tree method, shelterwood methods, and uneven-aged reproduction methods (Baker et al. 1996; Guldin and Loewenstein 1999). Mid-rotation thinning of mixed shortleaf pine-loblolly pine stands may also inadvertently discriminate against shortleaf pine especially in eastern Coastal Plain stands, because the more slowly growing shortleaf pine trees are more likely to be removed. There is a need to plant seedlings and encourage natural regeneration, as shortleaf pine seedlings are not as common as they could be. Oswalt (2011) used forest inventory and analysis data to show that large-diameter class trees dominate sites with significant shortleaf pine populations and that individuals of less than 1-in. diameter were very minor components.

Phylogeny

As mentioned, shortleaf pine is the most widely distributed southern yellow pine, a group of North American pines belonging to the subgenus *Pinus* section *Trifoliae* subsection *Australes*, which is distributed in the southeastern USA, eastern Mexico, and the islands of the Caribbean Sea. According to chloroplast DNA phylogenetics analysis, shortleaf pine's nearest relatives are Hispaniolan pine (*Pinus occidentalis* Sw.), Cuban pine (*Pinus cubensis* Grisebach), slash pine, longleaf pine, loblolly pine, and Caribbean pine (*Pinus caribaea* Morelet) (Hernandez-Leon et al. 2013). Within the most common southern yellow pines, shortleaf pine chloroplast DNA appears most similar to longleaf pine, a species with which it does not easily hybridize (Nance et al. 1991; Wagner et al. 1992). In nature, shortleaf pine most commonly associates with loblolly pine, with which it occasionally hybridizes (Tauer et al. 2012). During the Pleistocene glaciation, shortleaf pine apparently lived along the coast of the Gulf of Mexico, migrating northward when the glaciers receded about 14,000 ybp into their current range (Schmidtling 2007).

Cytogenetics and genomics

Like all pine species, shortleaf pine is diploid with 12 pairs of chromosomes ($2n = 2x = 24$, Mirov 1967) which correspond to 12 linkage groups (see for example Islam-Faridi et al. 2007b, Echt et al. 2011a, Westbrook et al. 2015). Many molecular markers, such as simple sequence repeats (SSRs), developed for use in loblolly pine function in shortleaf pine (Nelson et al. 2007), as the two species are close relatives (subsection *Australes*). The shortleaf pine genome is of similar size to other pine genomes. Shortleaf pine has a *C* value (a measure of the DNA content of a chromosome) of 21.73 pg measured by laser flow cytometry and 22.77 pg measured by scanning Feulgen microspectrophotometry (Wakamiya et al. 1993). These numbers translate to 21.4 and 22.5 Gb for the haploid genome, respectively. In comparison, the recently sequenced loblolly pine genome has *C* values of 21 to 23 pg. The loblolly pine reference genome sequence has 20.15 Gb and 50,172 validated genes (Zimin et al. 2014; Neale et al. 2014). Cytogenetic analyses of the major ribosomal DNA (rDNA) sites show shortleaf pine to be more similar to slash pine than to loblolly pine (Islam-Faridi et al. 2007a). All three of these species contain seven major rDNA sites, with one of these being heterologous with respect to rDNA quantity in both shortleaf and slash pines but not in loblolly pine or longleaf pine (Islam-Faridi et al. 2008).

At present, no efforts are underway to sequence shortleaf pine's genome, but a great deal of information about loblolly pine's genome will be useful in comparative genomics studies in shortleaf pine. Indeed, currently, there are only 150 shortleaf pine gene sequences listed in the National Center for Biotechnology Information (NCBI) database. Most of these (141) are from one expressed sequence tag (EST) library developed to compare gene expression of saplings following topkill in shortleaf pine and loblolly pine (Liu et al. 2011). The remaining nine sequences are from chloroplast genes (one from Hernandez-Leon et al. 2013; one from Kan and Guo 2011; four from Syring et al. 2005, direct submission; and three from Saich et al. 2005, direct submission). With the availability of the loblolly pine reference genome and increasingly less-expensive sequencing platforms, comparative genomics tools can and should be used to improve shortleaf pine genetics resources.

Provenance variation

The Southwide Southern Pine Seed Source Study (SSPSSS) was established in 1951 to evaluate the range-wide variability and viability of shortleaf pine, loblolly pine, longleaf pine, and slash pine seed sources (Schmidtling 2001). Wells and Wakeley (1970) examined 10-year-old plantings of shortleaf pine from the SSPSSS, employing 40 plantings of 23 seed sources across the shortleaf pine range.[1] The seed sources were divided into three series: latitude, longitude, and intermediate. The latitude series and the intermediate series were both designed to represent the north-south variation, though the latitude series encompassed a wider range than the intermediate series did. The longitude series represented an east-west transect across the shortleaf pine range. Since many of the plantings were originally established in the early 1950s, the drought from that era affected overall survival (Andreadis et al. 2005). The only provenance trend detected for survival was that trees from northern sources out-survived trees from southern sources in the northern plantings in New Jersey, Pennsylvania, and Missouri. Height (and by extension, volume) was the only other variable that significantly differed among provenances. The growth response was most significant in the latitude series. The results showed that southern sources outperformed northern sources in southern and central plantings, but the northern sources outperformed southern sources in northern plantings. While there was no longitudinal trend, trees from Ashley County, Arkansas (which is in the southeastern part of the state), consistently outgrew all other sources in the longitudinal series.

Posey and McCullough (1969) analyzed data collected from 10-year-old trees planted in two SSPSSS tests in southeastern Oklahoma. A test in Pushmataha County represented a north-south transect of seed sources, while the test in McCurtain County represented an east-west transect. In these plantings, western shortleaf pine sources had significantly better survival and eastern gall rust (caused by *Cronartium asclepiadeum* f. sp. *quercuum*) resistance than the eastern sources, and southern sources showed better growth in height and diameter than northern sources. While Posey and McCullough do not address it, the better survival of western sources may be associated with better drought adaptation, as well as better eastern gall rust resistance. Tauer (1980) revisited these trees 10 years later and found the same trends and also determined that western source trees had significantly greater wood specific gravity than the trees of eastern origin.

Gwaze et al. (2007B) measured shortleaf pine seed sources planted at two sites in Dent County, Missouri (central Missouri), as part of the SSPSSS. At age 30, trees from northern sources outgrew trees from southern sources, and trees from the New Jersey seed source performed better than trees from the local Missouri seed source. Additionally, trees from northern sources had better survival (62.2 % for the New Jersey sources) than did the trees from southern sources (23.5 %). These results agree with data from Wells and Wakeley (1970) indicating that northern seed sources are generally better adapted to northern sites than are southern seed sources. Tauer and McNew (1985) used seed from 15 widely

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[1] Contact author C. Dana Nelson for SSPSSS data going out to 40 years on some sites.

dispersed Oklahoma shortleaf pine stands in two plantings in eastern Oklahoma, one in the north and one in the south. After 10 years, they reported that there was no statistical difference across seed sources for most traits (height, DBH, straightness, and crown rating) but that stand survival was lower at the northern planting, probably due to more xeric conditions there.

Most of these past provenance surveys focused on determining optimal seed sources for achieving high survival and growth for various sites and assumed that climate would remain constant. As long-lived forest trees, shortleaf pines are well-adapted to climatic fluctuations, but those adaptations relate more for survival and not necessarily optimal growth. Future provenance tests should be linked to the latest climate change models to predict the best sources of shortleaf pine for planting in various regions. Fortunately, much of the data produced by previous provenance test studies can be used in climate change predictions (Matyas 1994). In addition to the temperature and moisture effects of climate change, survival and recovery following disturbances such as drought, fire, or ice storms may be more important than growth and volume production to maximize stand resiliency and to meet objectives related to ecosystem restoration.[2] Provenance testing generally has not explored these traits. Given the strong selection pressures of disturbances such as fire, differing regional and local disturbance regimes may have caused population differentiation.

Genetic diversity

Most generally, the term “genetic diversity” refers to the presence of genetic differences (variation) among members of a species or population. It is a good proxy for the health of a species, since genetically diverse species have a larger battery of potentially adaptive genes to use in response to changes and stress. Several studies of shortleaf pine diversity use molecular techniques to estimate different parameters of genetic diversity. So far, research has focused on population differentiation—the proportion of a species’ diversity that can be accounted for by the differences among populations and not within populations—which can be estimated with several statistics, including F_{ST}, G_{ST}, and Φ_{PT} (Table 1).. No known studies have focused on within-population differentiation in shortleaf pine.

The F_{ST} statistic can be calculated from the results of co-dominant genetic markers, such as isoenzymes and simple sequence repeat DNA markers (SSRs, also called microsatellites), while G_{ST} can be calculated from the results of dominant genetic markers like amplified fragment length polymorphism markers (AFLPs). The newer Φ_{PT} metric (Excoffier et al. 1992) can be calculated for either kind of data. All involve complex statistical models, though they work by different means. The F_{ST} is based on allele frequencies among populations (Meirmans and Hedrick 2011) or on an ANOVA approach (Weir and Cockerham 1984). The Φ_{PT} is also calculated using allele frequencies among populations, but it involves the use of Euclidean distances in matrices to avoid bias. In contrast to F_{ST} and Φ_{PT}, G_{ST} is based on relative heterozygosity and it has been criticized for certain biases in its results (Jost 2008). One possible bias is a tendency toward underestimation of population differentiation, especially when heterozygosity is generally high, which it is in pines. Population differentiation is a measurement of how isolated populations are to each other genetically. As a wind-pollinated, outcrossing forest tree with a largely contiguous range, it is expected that shortleaf pine will have a small amount of population differentiation (<~0.1) for selectively neutral genetic markers. Most studies have reflected that expectation. While differences among the reported values occur, there is not a tradition of reporting confidence intervals (using bootstrap methods) or estimates of standard errors (using jackknife methods), so determining real differences among the estimates is not possible.

Edwards and Hamrick (1995) estimated G_{ST} using 22 isoenzymes, which are polymorphic enzymes that can be differentiated and visualized on starch gels and serve as neutral genetic markers. The researchers used up to 48 individuals from 18 sites that extended from the eastern to western portions of shortleaf pine’s range. Generally, the sites represented the central and northern parts of the range, as the southernmost site was in southeastern Oklahoma. Population differentiation (G_{ST}) was ultimately estimated to be 0.026, indicating that most of shortleaf pine’s diversity comes from within the populations and not among them.

Raja et al. (1997) used 23 isozyme systems covering 39 loci on 126 trees from 15 sites to estimate F_{ST}. The sites used in the study were evenly spread across shortleaf pine’s geographic range. From this work, they estimated F_{ST} for shortleaf pine to be 0.089. The authors explain that their estimate for population differentiation is more than that of Edwards and Hamrick’s (1995) due to their study having more private alleles than the previous study. Private alleles are variants of markers that are not shared among populations, either because they originate in a population through mutation and they have been concentrated there through some evolutionary process such as local selection pressure or genetic drift. The site selection of Raja et al. (1997) also covers a greater north-south variation than did Edwards and Hamrick’s (1995), which may affect population differentiation, since provenance tests of shortleaf pine seed sources reveal that the trees are more variable according to north-south adaptation than to other factors (Wells and Wakeley 1970).

Two studies of trees from the SSPSSS used two different molecular techniques to estimate population differentiation in shortleaf pine. Xu et al. (2008b) examined 93 shortleaf pines representing a range-wide sample of the species from that study using AFLPs. This technique generates dominant

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[2] Contact author Barbara Crane for details.

Table 1 Results from studies that estimate genetic differentiation in shortleaf pine, including marker type used, number of loci (or alleles) scored, number of population (or sites) sampled, number of individuals sampled, type of statistic estimated, and the estimate for genetic differentiation

Study	Marker type	Number of loci	Number of populations	Number of individuals	Statistic	Estimate
Edwards and Hamrick (1995)	Isozymes	22	18	48	G_{ST}	0.026
Raja et al. (1997)	Isozymes	39	15	126	F_{ST}	0.089
Xu et al. (2008B)	AFLP	794	12	93	G_{ST}/Φ_{PT}*	0.153/0.057*
Stewart et al. (2010)	SSR	42	12	90	Φ_{PT}	0.080
Stewart et al. (2012)	SSR	25	10	151	Φ_{PT}	0.146

*The Φ_{PT} estimated for Xu et al. (2008B) comes from Stewart et al. (2010) but uses the Xu et al. (2008B) data

genetic marker data from pine DNA. A genetic marker technique is considered to produce dominant data when each locus can only be reported as having one allele (the dominant allele) present or absent. They estimated the population differentiation parameter G_{ST} to be 0.153, which is relatively high compared to other such estimates in this species but still represents a prolific outcrossing species. Stewart et al. (2010) reexamined the data from that study and estimated Φ_{PT} to be 0.057, which was in more agreement with previous studies. Stewart et al. (2010) also analyzed 90 trees from the SSPSSS representing a range-wide sample of the species with SSR markers. These markers generate codominant genetic data. They estimated Φ_{PT} to be 0.080. Stewart et al. (2012) used 25 SSR markers to test 151 shortleaf pine seedlings sampled from the same counties as the original SSPSSS, but 50 years later, and estimated Φ_{PT} at 0.146. Differentiation among the seed sources may have increased over the time due to the increased habitat fragmentation and the increase in shortleaf pine × loblolly pine hybridization.

Tree improvement

Shortleaf pine genetic improvement has been a fraction of that for loblolly pine, the tree that dominates the southern timber industry of the southeastern USA. The USDA Forest Service began its shortleaf pine tree improvement program in the 1960s, which included breeding and progeny testing. Shortleaf pine seed orchards were established in North Carolina, Mississippi, Louisiana, and Arkansas. The forest service installed 155 shortleaf pine progeny tests throughout the southern region in the 1980s (Crane 2014). They were assessed in 2012; survival rates averaged 65–95 %. Due to changing goals and missions within the forest service in the 1990s, breeding and progeny testing activities were ceased. However, all shortleaf pine first- and second-generation seed orchards are actively maintained for seed production—in support of reforestation, restoration, and surplus seed sales to state agencies.

In Oklahoma, shortleaf pine tree improvement began with the Oklahoma State University Department of Forestry in 1965 at the Kiamichi Forest Research Station. Professor Clayton Posey led the early tree improvement projects, which included shortleaf pine, as well as loblolly pine and eastern cottonwood. In 1980, these efforts were joined with those of the Western Gulf Forest Tree Improvement Cooperative (Lantz and McKinley 2003). At present, the Kiamichi Forest Research Station is co-owned and operated by Oklahoma State University and the Oklahoma Department of Agriculture, Food, and Forestry. Currently, the station does not conduct tree improvement but maintains a shortleaf pine seed orchard.

The Western Gulf Forest Tree Improvement Cooperative shortleaf pine breeding program (Lowe and van Buijtenen, 1990) produces first- and second-generation improved shortleaf pine seedlings for purchase from two entities. Bulk orders can be fulfilled by International Forest Company (Moultrie, GA), and smaller orders can be fulfilled by the Arkansas Forestry Commission (Little Rock, AR). In general, however, the efforts to improve shortleaf pine for planting are small compared to the improvement programs for loblolly pine. In the future, some areas, especially more xeric and northern places, could benefit from expanded shortleaf pine breeding.

Missouri and Arkansas have been collaborating on shortleaf pine improvement since the early 1960s in a breeding project on the Mark Twain National Forest in Missouri and the Ozark and Ouachita National Forests in Arkansas. The first seeds of the first-generation selections were collected in the early 1980s, and collections from the second-generation started in 2003. This program focuses on generating seed for shortleaf pine restoration of endangered ecosystems, especially for habitat related to the red cockaded woodpecker (*Picoides borealis*) (Studyvin and Gwaze 2007). This program is also part of a program that provides shortleaf pine seed for sale in Missouri (Gwaze et al. 2007A).

A number of agencies continue to actively maintain shortleaf pine seed orchard resources. These federal and state agencies include the following: USDA Forest Service, Tennessee Division of Forestry, Georgia Forestry Commission, Arkansas Forestry Commission, Oklahoma Forestry Services, North Carolina Forest Service, and Kentucky Division of Forestry (although seed has not been collected in some time). However, state forestry agencies in the southeastern USA are generally decreasing shortleaf pine genetic resources in response to budget cuts and priorities. In an effort to gather current shortleaf pine seed and orchard resources, the USDA Forest Service circulated a Shortleaf Pine Resources Survey in 2012 (see attached Appendix). Knowing the status of current resources will help in the planning process. The *Shortleaf Pine Initiative* (http://www.shortleafpine.net/) commenced in 2010 to enhance communication among shortleaf pine practitioners and partners and to further support shortleaf pine restoration and management activities.

Since tree improvement programs focused on shortleaf pine growth and productivity, they may have inadvertently included some shortleaf pine × loblolly pine hybrids. The lack of a basal crook and fast growth are desirable traits for tree breeders and seedling producers, but they are also traits that are typical for hybrid trees (Lilly et al. 2012). During selection, orchard managers may be inadvertently selecting for these traits in order to produce superior parents and families. Additionally, shortleaf pine seed orchards are generally located near loblolly pine orchards, increasing the opportunity for hybridization. Since artificial regeneration of this species is important to its future, it is critical that the genetic integrity of planting stock be maintained. Unlike shortleaf pine, hybrids do not generally re-sprout following topkill from fire (Bradley 2015). As yet, it is unknown whether hybrids maintain shortleaf pine's resiliency to disturbance, cold, and drought, so forest managers should be aware of this potential problem. However, more research is required to determine whether improved shortleaf pines have hybrid character or not. Stewart et al. (in review, *Forest Science*) used SSR markers to find that about 8 to 10 % of USDA Forest Service shortleaf pine seed orchard trees and 0 to 10 % of several state shortleaf pine seed orchard trees were classified as either F1 hybrid trees or F1 backcrossed into shortleaf pine by one generation.

Hybridization and introgression—artificial and natural

Artificial hybridization for tree improvement

Shortleaf pine is known to hybridize with several related pine species. Hybrids of shortleaf pine and slash pine may outperform either species on dry sites (Schmitt 1968). Hybrids of shortleaf pine and loblolly pine, as well as backcrosses, have been relatively easy to produce (Schmitt 1968). Shortleaf pine also has been crossed with longleaf pine and even a hybrid of longleaf and loblolly pines (Sonderegger pine (*P. palustris* × *taeda*)) (Chapman 1922; Snyder and Squillance 1966). Of these hybrids, two have received attention for their possible commercial value: shortleaf pine × slash pine hybrids and shortleaf pine × loblolly pine hybrids and their backcrosses to slash pine (Nelson 1991) or loblolly pine (La Farge and Kraus 1977, 1980; Kraus 1986), respectively.

Shortleaf pine × slash pine hybrids appear to have hybrid vigor—that is, they outperform their parent species—in their growth performance (Schmitt 1968; Wells et al. 1978). Also, their resistance to fusiform rust (*C. quercuum* f. sp. *fusiforme*) is similar to the rust resistance of the shortleaf pine parent and not the susceptible slash pine parent (Schmitt 1968; Kraus and Powers 1984). Past work also emphasized seed production of what was believed to be a possibly "promising" improved tree (Wakeley et al. 1966). Backcross generation hybrids with slash pine as the recurrent parent showed overall promise in growth rates, but rust resistance was largely controlled by the recurrent parent (Nelson 1991). However, little work with this hybrid has continued today, and no natural shortleaf pine × slash pine hybrids have been reported.

Shortleaf pine × loblolly pine hybrids are considerably more important for breeding and ecological reasons than shortleaf pine × slash pine hybrids. The earliest report of artificial hybrids was made by Schreiner (1937), who made successful crosses at the Institute of Forest Genetics in Placerville, California. First-generation shortleaf pine × loblolly pine crosses have many traits that are intermediate of the two parent species, including the sizes of needles and cones and the number of needles per fascicle. Loblolly pine generally has three needles per fascicle—and sometimes two, four, or five—and shortleaf pine has two or three needles per fascicle. The hybrids have two or three needles per fascicle but have more three-needle fascicles than shortleaf pine (Little and Righter 1965; Snyder and Hamaker 1978).

Hybrid performance varies by study. Benson et al. (1982), as well as Schultz (1997), reported that the hybrids were intermediate to their parent species in growth and survival. Schoenike et al. (1977) indicated that hybrids (which were identified by morphology) have intermediate growth to loblolly pine and shortleaf pine. Other studies have reported more dominance of loblolly pine traits. Mergen et al. (1965) observed the hybrids as being more like loblolly pine (that is, having faster growth). Sluder (1970) reported that hybrids grew about as quickly as loblolly pines. Lilly et al. (2012) and Will et al. (2013) also found that the hybrid saplings grew as quickly as loblolly pine saplings but maintained the superior leaf-level water-use efficiency of shortleaf pines. The hybrids in their study also lacked the strong basal stem crook found in shortleaf pine saplings, a trait with is thought to increase fire resistance.

Another major advantage of shortleaf pine × loblolly pine hybrids is their disease resistance. Shortleaf pine is susceptible to littleleaf disease (caused by *Phytophthora cinnamomi*), and loblolly pine is susceptible fusiform rust (caused by *C. quercuum* f.sp. *fusiforme*). Interestingly, western trees of both species are more resistant to their respective pathogens (Bryan 1973; Squillace 1976), a trend that may be related to natural introgression (Hare and Switzer 1969). Schoenike et al. (1977) found that hybrids grown in the Piedmont of South Carolina were resistant to both littleleaf disease and fusiform rust, and

Benson et al. (1982) reinforced these results. Other studies also showed that hybrids are resistant to fusiform rust, similar to their shortleaf pine parents (La Farge and Kraus 1977, 1980; Florence and Hicks 1980; Kraus et al. 1982; Kraus 1986).

Natural hybridization with implications for conservation

Zobel (1953) was the first to propose that loblolly pine × shortleaf pine natural hybrids occur. He observed numerous trees in Texas that appeared to have traits intermediate to shortleaf pine and loblolly pine. At the time, natural hybrids of loblolly pine and longleaf pine were well-known (Chapman 1922) and relatively common (Zobel 1953), and there was interest in hybrid pines for tree improvement. At first, identifying natural hybrids could only be accomplished through morphological measurements. While morphological data are easy to acquire, traits are subject to complex genetic and environmental effects. Nonetheless, researchers generated several studies of loblolly pine × shortleaf pine hybrids using morphological measurements. Mergen et al. (1965) found 10 out of 40 trees sampled from Harrison County, Mississippi, and 4 out of 22 trees sampled from Greene County, Mississippi, were likely hybrids according to analysis with a suite of morphological characters. They did note that there was a great deal of variation of characters within species as well. Hicks (1973) proposed that research using morphology proceed with the use of six traits: needle length, fascicle sheath length, number of needles per fascicle, terminal bud width, cone length, and seed weight, but he did not identify any hybrids in his study. Abbott (1974) conducted a hybrid study based on morphological measurements after he found an “atypical” tree in an Oklahoma State University seed orchard. In his thesis, 19 loblolly pine and 12 shortleaf pine selections were studied using a hybrid index that incorporated needle length, number of needles per fascicle, cone length, seed weight, and fascicle sheath length. One loblolly selection and three shortleaf pine selections were hybrid, according to his index. Cotton et al. (1975) and Schoenike et al. (1977) both used morphological measures to search for natural hybrids in Texas and North Carolina, respectively, but neither found obvious hybrids.

Advancements in biochemistry gave geneticists more tools for the identification of hybrids. Hare and Switzer (1969) found protein banding evidence that some loblolly pine trees from Texas may have some shortleaf pine genetics. They concluded that this hybrid character may explain why loblolly pines from the region exhibit greater fusiform rust resistance than pines in the east. Florence and Hicks (1980) examined 29 loblolly pines, shortleaf pines, and suspected hybrids (identified by morphology) using protein banding patterns on acrylamide gels. Their protein banding patterns generally confirmed the morphological classifications.

Later, even more advanced isoenzyme (also called isozymes or allozymes) techniques were employed to identify hybrids. Edwards and Hamrick (1995) and Raja et al. (1997) both identified hybrids using isoenzyme techniques in shortleaf pine populations. Both studies found that hybrids between shortleaf pines and loblolly pines were more prevalent in the west than in the east. Edwards and Hamrick (1995) reported 4.6 % of shortleaf pines west of the Mississippi River were hybrids and 1.1 % of the shortleaf pines east of the Mississippi River were hybrids. They relied on one isoenzyme marker (the isocitrate dehydrogenase, or IDH, marker) to identify their hybrids. Note that by using only this marker, they could only identify F1 hybrids and half of all backcrosses by the rules of Mendelian genetics. Raja et al. (1997) indicated that the hybridization rate was higher than that, classifying 16 % of the shortleaf pines west of the Mississippi River as being hybrids and 4 % of shortleaf pines east of the Mississippi River as hybrids. Their study employed 39 loci, and the range of sampled trees extended further south than did the range used by Edwards and Hamrick (1995), but the estimates for the hybrid frequencies were only based on the IDH marker. Chen et al. (2004) used isoenzymes and a chloroplast DNA marker (which identifies the paternal parent species) to identify hybrids in Montgomery County, Arkansas, where the sympatric range of loblolly pine and shortleaf pine transitions into the allopatric range of shortleaf pine. They found 12.5 % hybrid pines in the shortleaf pine population, some of which were in the shortleaf pine allopatric range and some of which were not heterozygous for the IDH marker, indicating that genes were being transferred over generations or over distances via loblolly pine pollen.

DNA-based genetic markers have displaced isoenzymes as the leading method for genotyping pine trees because they are less expensive, technically simpler, and more abundant. Xu et al. (2008a) used AFLPs and the IDH marker to determine the hybridization rates in 93 shortleaf pine and 102 loblolly pine trees from the SSPSSS, trees representative of geographically diverse populations from the 1950s. They found that the hybridization rate between these two species was 16.3 and 2.4 % in shortleaf pine populations west of and east of the Mississippi River, respectively. In the loblolly pine trees of the study, the rate was 4.5 and 3.3 % west and east of the Mississippi River, respectively. These results support the theory that hybridization is more common in the west than in the east. Stewart et al. (2010) followed up on Xu et al. (2008a) to test the same trees (though three shortleaf pine trees were omitted from the dataset due to degraded DNA) with the SSR marker method, employing 42 markers, plus the IDH marker. They found that the hybridization rates were 7.5 % in shortleaf pine west of the Mississippi River and 0 % in shortleaf pine east of the Mississippi River. In loblolly pines, 9.1 % of the trees west of the Mississippi River were hybrids and 3.3 % of the trees east of the Mississippi River were hybrids.

To test for changes in the hybridization rate between the SSPSSS population collected in the 1950s and young, naturally regenerated loblolly and shortleaf pine from modern stands, Stewart et al. (2012) used 25 SSR markers to test 165 loblolly pine saplings and 151 shortleaf pine saplings sampled from the same counties that the trees from the SSPSSS originated. They found that the hybridization rate (which included trees classified as first generation hybrids and backcrosses out to the third generation) in shortleaf pine stands had increased to 54 % in the west and 40 % in the east. The rates in loblolly pine stands had increased to 20 % in the west and 29 % in the east. These increases are alarming, because introgression—the process of genes moving from one species to another through hybridization and backcrossing—is a known cause of extinction (Allendorf et al. 2001). Given the previously reported hybrid vigor, it is likely that fire exclusion over the last 60 or more years may be allowing hybrids to encroach into shortleaf pine habitat because the hybrids lack the strong basal stem crook that is thought to give shortleaf pine seedlings and saplings increased fire tolerance (Lilly et al. 2012; Will et al. 2013). Hybrid seedlings and saplings can be excluded from sites with a frequent fire regime. Stewart et al. (2015) showed that a 3-year fire interval nearly eliminated loblolly pine and loblolly pine × shortleaf pine seedlings from a pine savanna system in the Tall Timbers Research and Land Conservancy Center (located in northern Florida), while neighboring unburned patches maintained a mix of shortleaf pine, loblolly pine, and their hybrids. Bradley (2015) used planted loblolly pine, shortleaf pine, and artificially produced shortleaf pine × loblolly pine F1 hybrid pines to find that surface fire killed all 1- and 2-year-old seedlings of loblolly pine and hybrids, while approximately half of the topkilled shortleaf pine resprouted from the basal crook. Forest managers who wish to prevent hybrid encroachment into shortleaf pine ecosystems should employ prescribed fire often enough to eliminate hybrids seedlings and saplings before they otherwise become large enough to survive surface fire, that is before the foliage and terminal buds remains above the flames and the bark becomes think enough to insulate the cambium.

In addition to fire exclusion allowing the survival and proliferation of loblolly pine × shortleaf pine seedlings, widespread establishment of loblolly pine plantations may be increasing the incidence of cross-pollination (Tauer et al. 2012). Stewart et al. (2013) conducted a case study of hybrids using 25 SSR markers on nearly 400 shortleaf pine saplings and nearly 100 adult shortleaf pine trees spread across four sites in the Caney Creek Wilderness Area in the Ouachita National Forest in Arkansas, which is located just within the allopatric range of shortleaf pine. The stands were different distances downwind of extensive loblolly pine plantations, ranging from 100 m to 10 km away from the nearest plantation. They found that hybridization was higher in the stand nearest to the plantation than in the stand furthest away. They also found a higher percentage of hybrids in the parent trees than in the progeny, indicating that hybridization had been going on for some time at the site and that the hybrids may be selected for in the long run.

Given that introgression with loblolly pine may be a threat to shortleaf pine, further study on the phenomenon should be encouraged. The development of a fast DNA-based hybrid identification system could help foresters find where hybridization is occurring in their forests. A better understanding of what factors lead to natural hybridization could help managers better administer their lands for maintaining shortleaf pine's genetic integrity. Additional information about where in the shortleaf pine range hybrids are the most serious problem will also help strategic planning in the forests of the southeastern USA.

Past human activity probably has increased the level of hybridization between loblolly pine and shortleaf pine, and forest managers should be aware of these effects in weighing their options for managing shortleaf pine in an ecologically sustainable way. Forest managers should consider terrain, climate, fire regime, and other local conditions, which may affect the process of hybridization. Guldin (2007) states that fire is needed to restore shortleaf pine, and fire may also serve to remove most hybrids and help retain the genetic integrity of shortleaf pine. To that end, reintroduction of fire as a management tool is a critical part of the future of successful shortleaf pine management. If so, it may be increasingly difficult to manage areas with fragmented ownership or near higher human populations as prescribed fire becomes more difficult to employ.

The rapid increase in loblolly pine × shortleaf pine hybrids since the 1950s implies perturbations to the ecological interactions that previously maintained the genetic integrity of both species. Shortleaf pine is particularly at risk, because it originates mostly from natural regeneration, unlike loblolly pine with over 12 million ha of plantation in the southeastern USA (Fox et al. 2007). While loblolly pine × shortleaf pine hybrid seedlings are readily establishing, currently, they may have trouble persisting and regenerating in a future climate with longer and more frequent droughts as well as increased risk of wildfire. Replacement of pure shortleaf pine with hybrids may reduce the resiliency of southern pines to future disturbances and climate regimes and may eventually result in extirpation of the pine component from areas of the southeastern USA.

Land management efforts should be undertaken to conserve the genetic integrity of shortleaf pine and preserve important traits not found in the faster growing hybrid and loblolly pine genotypes. Assuming that larger hybrid pines (>10- to 15-cm ground line diameter) are resistant to topkill from fire in a manner similar to loblolly pine, the window of opportunity to kill hybrids is the seedling or sapling stage. Waiting too long may produce an irrevocable change, resulting in permanent loss of shortleaf pine from large areas and a less resilient forest.

Summary and conclusions

Shortleaf pine is an important forest tree species for both silvicultural and ecological reasons. Silviculturally, it is a valuable timber species to both large and small woodland owners, although by the nature of its distribution and silvical properties, it tends to be most favored by non-industrial private landowners and publically managed lands using natural regeneration and mixed species stand composition. Ecologically, for the same distribution and silvical reasons, it is a valuable mid- to late-successional species, comprising a major component in shortleaf pine, shortleaf pine-oak, and loblolly pine-shortleaf pine cover types and a lesser component of 15 other Society of American Foresters defined cover types (Lawson 1990). In addition, it is a foundational species in savanna ecosystems, such as the shortleaf pine-bluestem ecosystem, which have declined across the southeastern USA largely due to fire exclusion. The overall decline in these shortleaf pine-influenced ecosystems over the last several decades has motivated the natural resource conservation community to promote increased cultivation and planting of shortleaf pine in hopes of reversing this trend. For example, the USDA Forest Service has undertaken efforts to restore approximately 100,000 ha of shortleaf pine—bluestem savanna on the Ouachita National Forest and has recently initiated similar efforts on other forests such as the Ozark and Mark Twain National Forests. Shortleaf pine restoration in the Southern Appalachians is also underway, as those ecosystems have been severely degraded. In addition, the Shortleaf Pine Initiative (www.shortleafpine.net) recently started to focus on restoration of shortleaf pine on private as well as public lands.

Understanding the genetic basis of adaptation to various environments and the potential for adapting to new environments is an important research topic both in the past but even more so today and into the future. Past studies and findings have been reviewed here. Primary among them are the provenance tests, especially the SSPSSS, that describe a species with large amounts of adaptive trait variation distributed in largely clinal patterns along temperature and moisture gradients. This has led to practical recommendations for seed collecting and planting zones as described by Schmidtling (2001). These provenance testing results also indicate that shortleaf pine has great potential for adapting to future environments given proper maintenance and management of genetic diversity.

Genetic diversity as measured by neutral molecular markers was also found to be high, with a significant amount of diversity maintained within local areas and substantial gene flow between areas (Xu et al. 2008a, Stewart et al. 2010). However, more recent research contrasting shortleaf pine populations from the 1950s and the early 2000s has shown a couple important trends (Stewart et al. 2012). First is a reduction in gene flow among areas as measured by increased genetic differentiation among areas. Second, there is a large increase in the proportion of hybrid types across the whole of the range, but especially in the areas west of the Mississippi River. Both of these findings will need to be reconciled when planning reforestation, restoration, and assisted migration plantings and programs. Already, the USDA Forest Service's southern region is genotyping seed orchard clones and seedlots in an attempt to eliminate the hybrid types from making further contributions to the next seed orchard seed crops and forest tree nursery seedling crops. In some orchards this can mean the removal of up to 10 % of the parental clones (Stewart et al. submitted, *Forest Science*). In addition, it will be important to maintain connectivity among reservoir populations of diverse shortleaf pine and new restoration plantings, and a series of restoration seed reserves (RSRs, Echt et al. 2011B) are recommended to maintain genetic diversity and adapted potential far into the future.

Future genetics research should focus on five areas: (1) improving molecular tools for better understanding and managing genetic diversity and for utilizing shortleaf pine's adaptive traits and genes in related species, especially through the development of new and well-defined markers; (2) developing an understanding of shortleaf pine genetic diversity at more localized levels; (3) improving the understanding of the genetic architecture and physiological mechanisms of shortleaf pine's adaptive traits; (4) improving shortleaf pine as a species for silvicultural purposes; and (5) developing shortleaf pine RSRs for long-term genetic diversity maintenance and adaptive trait improvement.

Current assessments of the levels of genetic diversity and the status of hybridization and introgression in shortleaf pine rely on a couple dozen or so SSR markers and the prevailing software packages that handle this type of data with respect to these questions. These results have largely supported previous studies with earlier generation molecular techniques but there have been some new findings that were not possible to access before, such as quantitatively estimating genome proportions in hybridizing species. Based on this and what we have seen in other systems, we can anticipate additional new findings of importance for population management for tree improvement and gene conservation as we increase the potential for analyzing larger proportions of the shortleaf pine genome with higher resolution SSR scans, genome-wide SNP analyses, or even genotyping-by-sequencing (GBS). Toward this end, we see the generation of genome-level sequence data (both whole genome DNA and RNAseq) as a high priority for molecular-based genetics research. It is an opportune time to employ larger-scale genotyping with next-generation sequencing (NGS) technologies drastically lowering the price per million base pairs (Mbp) of data and with the availability of the reference genome for the closely related loblolly pine (Neale et al. 2014). Having the loblolly pine reference genome sequence greatly improves the value of any large-scale sequence data that can be generated for shortleaf pine.

To date, genetic diversity studies have covered on the entire shortleaf pine range or large regions of it. Measuring diversity on a finer scale could help us better understand the dynamics of pollination, natural selection, responses to known local stresses, and the impact of management. This work should be conducted in concert with building our knowledge of shortleaf pine genomics, since the markers used in diversity measurement could relate to genes or other loci that contribute to adaptation or other traits.

Among the southern pines, shortleaf pine is the only species that possesses the basal crook, a presumed adaptation to resprout following topkill from fire. An understanding of the importance of the basal crook, its heritability, and expression based on environmental influence is needed to refine seed source selection, nursery management, planting, and prescribed fire recommendations. Prescribed fire is used extensively as a cost-effective and efficient silvicultural tool to maintain shortleaf pine ecosystems (Zhang et.al. 2012); hence, the importance of basal crook resprouting. Additionally, prescribed fire can eliminate unwanted competition in the stand to favor shortleaf pine and give it the competitive advantage (Guldin 2007). A better understanding of shortleaf pine's drought tolerance and its genetic basis in regards to specific physiological traits, e.g., osmotic adjustment, water use efficiency, and morphological traits, e.g., root depth, root/shoot ratio, should be further explored to direct selection of seed sources with sites to maximize resilience in potentially future, drier climate. This new knowledge will facilitate ecosystem management recommendations and restoration guidelines being developed for assisted migration under climate change scenarios. Shortleaf pine, because of its flexible tolerance for a variety of sites (as evidenced by its extensive range), will most likely adapt better than most other southern pines to climatic variability. Increased understanding of its unique niche in the ecosystem is needed.

While loblolly pine has been the most important component of softwood timber production and tree improvement practice over the past several decades, shortleaf pine is still used silviculturally both as a mixed forest product in naturally regenerated coastal plain stands and as a high-value sawtimber product, especially in the Ouachita and Ozark Mountains west of the Mississippi River. Therefore, it should remain a priority in tree improvement. In particular, shortleaf pine covers the most extensive range of the southern pines, and it has some very useful and distinctive traits. These two general features make it a valuable species for mitigating and adapting to climate change and providing a useful potential resource for improving other conifer species. Among shortleaf pine's superior qualities and important adaptive traits, we note its 400-year potential lifespan; the potential for superior wood, given its slower growth rate; good resistance to drought, ice, and cold; resistance to some diseases that plague other species like loblolly and slash pine; and adaptation to frequent fire. For these reasons, we argue that it should be improved for silvicultural as well as conservation purposes. In addition, several of these traits could be transferred to other species through inter-species hybridization and molecular-assisted introgression, or even genetic engineering, as both have been proposed and accomplished for many crop plant species.

The primary emphasis of shortleaf pine genetics for restoration and management should focus on genetic diversity and species integrity. Shortleaf pine ecosystems were severely depleted by logging and displaced by agriculture and other forest systems over the last 150 years. The continued effects of land development, land conversion, and fire exclusion will have persistent negative impacts on this once plentiful, foundational species. The genetic diversity, represented by the multiple seed zones and ecotypes, needs to be maintained to support successful restoration across multiple sites. Currently, over 200 ha of improved shortleaf pine seed orchards are managed in the southeastern USA, but not all seed zones are represented. Seed harvested from these orchards provides the most suitable and durably adapted seed that will ensure resiliency when matched to the appropriate site and will help to ensure long-term restoration success. However, good cone crops only occur every 5–7 years, and although shortleaf pine seed remains viable in storage for 10–15 years, a lack of seed availability can be a constraining factor to restoration through planting. By maintaining and supplementing the genetic diversity, shortleaf pine will retain its biological plasticity in adapting to climate change. Typical shortleaf pine rotations can span 100 years and more, so planting the appropriate seed source is critical for sustainability. In addition, species integrity needs to be maintained, because introgression with loblolly pine poses a substantial genetic risk to shortleaf pine. Shortleaf pine has unique adaptive traits which may be lost or diminished by introgression which would further impair the effort to restore diverse, well-adapted sources of shortleaf pine. Restoring shortleaf pine ecosystems with shortleaf pine of high genetic integrity and diversity is of primary importance. One promising approach to species and gene conservation for restoration is the concept of RSRs (Echt et al. 2011b), which are similar to seedling seed orchards, but focus on gene conservation. Shortleaf pine RSRs would provide archives of genetic diversity that promote gene flow and could limit inter-species hybridization and introgression. A set of RSRs would represent the important environmental conditions (both existing and future) for shortleaf pine, ensuring continuing long-term adaptation for the species.

Compliance with ethical standards

Data archiving statement This manuscript contains no new data for public use.

Appendix

Shortleaf pine resources survey Tree improvement capacity: this section is to assess the capability of the tree improvement operation at the following location to meet the current and projected demand for genetically improved shortleaf pine seed.

Statement			
Status of Seed Orchards	**ACRES**		
First Generation			
Second Generation			
Advanced Generation			
Seed Production Areas			
Current Shortleaf Pine Seed Orchard Management	**YES**		**NO**
Original orchards retained, not managed, no seed collected			
Orchards retained, not managed, some seed collected			
Orchards retained, limited management, seed collected			
Orchards retained, actively managed, seed collected			
Orchards retained, actively managed, seed collected, additional genetics work underway or planned			
Orchards removed			
New orchards recently established on _______ acres			
Never had shortleaf orchards			
Seed Inventory	**POUNDS/ 1st or 2nd GEN SEED?**		
Approximate annual seed collection (averaged for last five years)			
Approximate seed in storage			
Seed Age			
Program Intentions Next Three Years	**YES**		**NO**
Maintain status quo			
Increase management intensity and seed collection activity			
Discontinue shortleaf efforts, remove orchards			
Mothball orchards for the time being			
Kiln Facility (Write in YES or NO and LOCATION)			

Geographic sources for genetic material in TI program: ____________________

Geographic area where seed/seedlings are adapted for out-planting: _______________

Additional Comments:

Survey Completed by (Name and Title):

Date:

Contact Information (Phone and Email):

Name of Facility:

Location:

Agency Name:

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