Hybridization in Naturally Regenerated Shortleaf Pine as Affected by the Distance to Nearby Artificially Regenerated Stands of Loblolly Pine

John F. Stewart, Charles G. Tauer, James M. Guldin, and C. Dana Nelson

ABSTRACT

The natural range of shortleaf pine encompasses 22 states from New York to Texas, second only to eastern white pine in the eastern United States. It is a species of minor and varying occurrence in most of these states usually found in association with other pines, but it is the only naturally occurring pine in the northwestern part of its range in Oklahoma, Arkansas, and Missouri. Over the whole of this area, it is apparent that shortleaf pine hybridizes with loblolly pine, including areas of species overlap (sympatry) and nonoverlap (allopatry). In this study, we used 25 DNA markers to measure the levels of hybridization and introgression in populations of juvenile and mature shortleaf pine from a wilderness area within the allopatric range of shortleaf pine that bordered loblolly pine plantings. Frequency of hybrids ranged from 9.2 to 24.0% among the populations sampled, and we found that levels of hybridization correlates with distances to the nearest loblolly pine plantations. However, the rates of hybridization and introgression apparently remained the same among the parent and juvenile populations.

Keywords: shortleaf pine, loblolly pine, introgression, molecular markers

Shortleaf pine (*Pinus echinata* Mill.) is an important forest tree species found throughout the southeastern United States. The species is known to hybridize with loblolly pine (*P. taeda* L.), another very important forest species in the southeastern United States (Hare and Switzer 1969, Zobel 1953, Hicks 1973). While the two species often occur together, loblolly pine generally occurs on more mesic sites, and shortleaf pine is generally found on more xeric sites. Both species are known to be early succession colonizers (Baker and Langdon 1990, Waggoner 1975). However, in the mid-South, the natural range of loblolly pine ends at the northern edge of the upper-western Gulf Coastal Plain. As a result, shortleaf pine is the only naturally occurring pine in the Ouachita and Ozark Mountains of Arkansas and Oklahoma, where it often forms pine-dominated stands, especially on southern aspects. This transition is not sudden, and local geography in the transition from mixed loblolly pine and shortleaf pine stands to shortleaf pine stands without loblolly pine is occasionally patchy. Additionally, loblolly pine is the plantation species of choice, including in locations outside of loblolly pine's natural range because of its superior growth rate relative to shortleaf pine. However, shortleaf pine has superior drought tolerance to loblolly pine (Lawson 1990), and it can generally withstand ice damage better than other southern yellow pines (Williston 1974).

Natural hybridization between these two species has been measured in a number of ways, including morphologically (Mergen et al. 1965, Cotton et al. 1975, Hicks 1973), using isozymes (Huneycutt and Askew 1989, Raja et al. 1997, Chet et al. 2004, Edwards-Burke et al. 1997), and using DNA-based markers (Xu et al. 2008, Stewart et al. 2010, Stewart et al. 2011). The latter two means of measuring hybridization are considered to be more accurate, because morphology can be affected by the environment in addition to genetic heritage. Modern DNA-based markers quickly and easily provide data for many types of genetic studies. Markers associated with specific genes or other DNA sequences that are randomly distributed within a species' genome can be used to generate large data sets of populations. In turn, these data are useful for determining diversity, geographic patterns of heredity, interspecific hybridization, and other parameters of the species of interest. It is a relatively inexpensive way to learn otherwise hidden details of ecological and economic importance.

In studies on hybridization between loblolly pine and shortleaf pine, more hybridization has been observed west of the Mississippi River than east of it (Edwards and Hamrick 1995, Xu et al. 2008, Stewart et al. 2010). Stewart et al. (2010) used microsatellites, a highly informative class of DNA markers (Guichoux et al. 2011), and found that 3.3% of loblolly pine were hybrids east of the Mississippi River, while west of the river, 9.1% of the trees had hybrid character. Similarly, for shortleaf pines, 0 and 7.5% of them were hybrids east and west of the Mississippi River, respectively. Most likely, the phenomenon of more hybridization in the western region was due to the more variable climate there, as weather affects the

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This article uses metric units; the applicable conversion factors are: meters (m): 1 m = 3.3 ft; kilometers (km): 1 km = 0.6 mi.

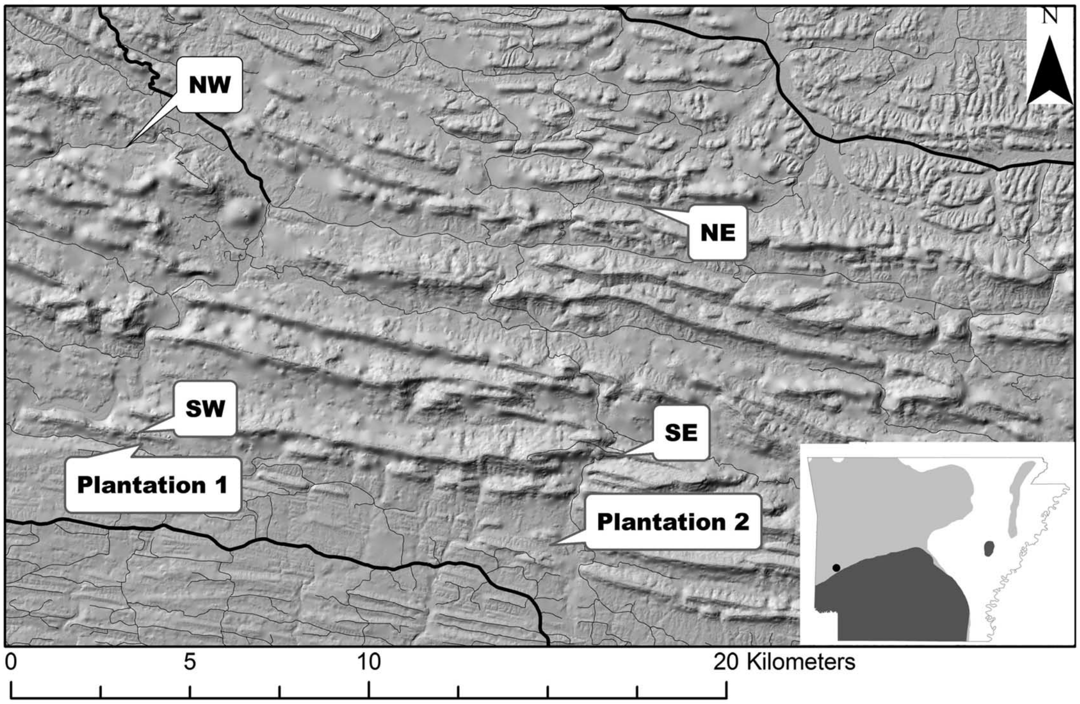

Figure 1. Map of collection sites. Needles from the parent populations and the juvenile populations were collected from the sites shown above: NW, SW, NE, and SE. The NW, NE, and SE populations were collected along roadsides, while the SW population was collected along a power line cut. When calculating distances for the distance to the nearest plantation and the distances among populations, the approximate sample transect center was used to estimate distance from sample location to plantation location. The site Plantation 1 is the nearest part of a loblolly pine plantation to the NW and SW sites, and the site Plantation 2 is the nearest part of a loblolly pine plantation to the NE and SE sites. The heavy lines indicate state or federal highways, and the lighter lines indicate minor roads. The dot in the insert represents the plot location within the state of Arkansas with the sympatric range in dark gray and the allopatric range of shortleaf pine in light gray.

timing of pollen shed and strobili receptivity. Since the 1950s, hybridization between loblolly and shortleaf pines has increased dramatically across the Southeast (Stewart et al. 2011). Stewart et al. (2010) and Stewart et al. (2011) showed increases in the hybridization rates in stands of both species. From the 1950s to 2007 and 2008, the rate of hybridization in shortleaf pine stands increased from 3.3 to 45.7%, and it increased from 4.5 to 27.3% in loblolly pine stands. These changes indicate a perturbation of the ecological barriers to hybridization that had previously existed.

In this study, we used DNA-based microsatellite markers to measure levels of hybridization and introgression in naturally regenerating shortleaf pine stands in the Caney Creek Wilderness Area located on the Ouachita National Forest in west-central Arkansas. This area is on the border of the allopatric range of shortleaf pine—that is, the part of the range of shortleaf pine in which loblolly pine does not also occur. However, extensive plantations of loblolly pine have been established immediately across the borderline road south of the Wilderness Area over the past 4 decades on private lands managed intensively for timber and fiber production in support of local forest products industry, and prior to the plantations, that land was mixed shortleaf pine and loblolly pine. The site represents a microcosm for the potential of loblolly pine pollen to fertilize naturally regenerating shortleaf pine stands. We hypothesized that the rate of hybridization would increase with proximity to the loblolly pine plantations. Understanding how the distance of shortleaf pine forests from loblolly pine stands affects the rate of hybridization is important to the future management of shortleaf pine.

Materials and Methods

Needle Source

Current-year needles were collected from four sites in the Caney Creek Wilderness Area, Polk County, Arkansas, which is part of the Ouachita National Forest (Figure 1). Each collection location was on the corner of an approximate rectangle about 32 km (20 miles) from east to west and 24 km (15 miles) from north to south. Collection sites were thus labeled northwest (NW), southwest (SW), northeast (NE), and southeast (SE). Sample sites were approximately 400 m transects about 50 m wide, along roads, except for the SW site, which was along a power line clearance. Twenty-five possible parent trees—trees that were at least 30 years old and labeled NWp, SWp, NEp, and SEp, depending on the source site—were sampled from each site, and 100 naturally regenerated juveniles, which were labeled NW, SW, NE, and SE, were also sampled from each site. The eight populations were defined as juvenile or mature trees from each collection site. No loblolly pine trees capable of producing cones were observed in the collection areas. Needles were collected in fascicles and shipped on ice to the forest genetics laboratory at Oklahoma State University (OSU), where they were stored at −70° C. On collection, number of needles per fascicle was counted for 35 fascicles per individual. In further analysis, the mean needles per fascicle was used for each individual.

DNA Extraction and Microsatellite DNA Markers

The DNA was extracted from leaf tissue by cutting the needles into small pieces and then using the Qiagen DNeasy 96 Plant Kit

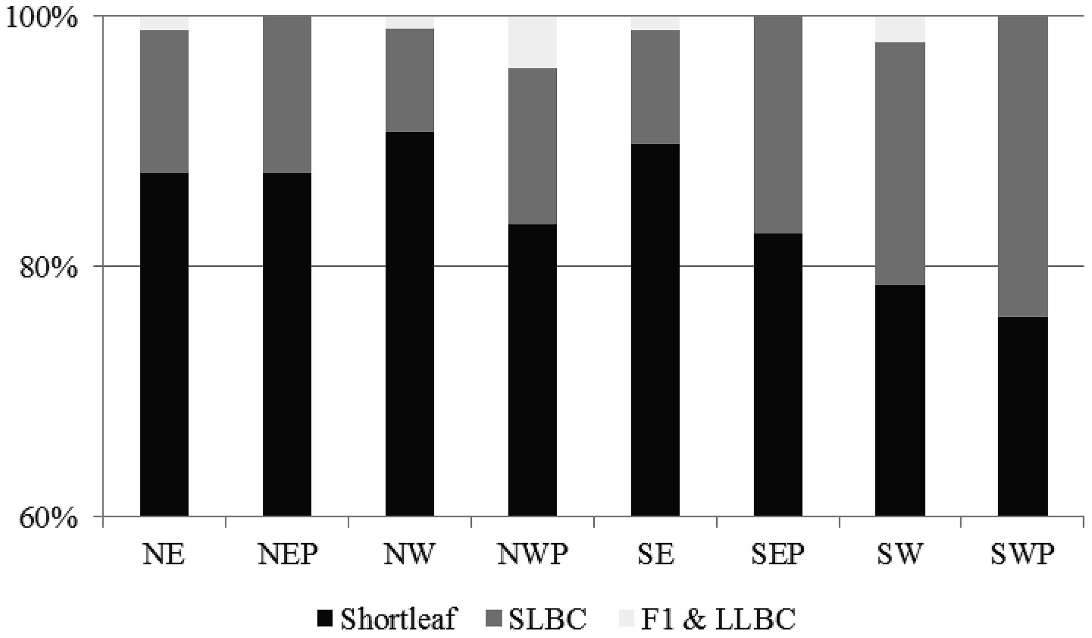

Figure 2. The hybridization rates in each population. NWP, NEP, SWP, and SEP are from representative parent populations, and NW, NE, SW, and SE are juvenile populations. Categorization of the individuals is based on *Q* values generated by Structure. F1 & LLBC trees are trees with *Q* values between 0.375 and 0.953. Shortleaf backcrossed trees (SLBC) are trees with *Q* values between 0.047 and 0.375. Trees with *Q* values less than 0.094 are categorized as shortleaf pine.

(Qiagen, Velencia, CA). Twenty-five microsatellite markers were used in this study. Of these, 11 had been previously confirmed to be polymorphic in shortleaf pine (Nelson et al. 2007): PtTX3052, PtTx3034, PtTX4205, RIPt0619, RIPt0629, PtTx3013, RPtest9, RIPt0079, RIPt0211, RIPt0388, and PtTx2123. Twelve were new markers developed for loblolly pine (Echt et al. 2011): PtSIFG_0437, PtSIFG_1207, PtSIFG_0424, PtSIFG_0265, PtSIFG_1190, PtSIFG_1295, PtSIFG_1008, PtSIFG_1166, PtSIFG_0493, PtSIFG_1018, PtSIFG_0440, and PtSIFG_0587. Two were tested and selected in the OSU lab from those provided in Chagne et al. (2004): SsrPt_BF778306 and SsrPt_ctg4487b.[1] Polymerase chain reaction and gel electrophoresis conditions are provided in Stewart et al. (2010). Allele names were standardized using reference samples and a scoring system similar to that described in Deemer and Nelson (2010).

Population Genetics and Hybrid Analysis

For all analyses, trees with more than 5 missing data points for the 25 markers were excluded. This accounted for 22 trees approximately evenly mixed across the collection locations. One tree that was later determined to be loblolly pine was also removed from the counts. Structure, version 2.3.2 (Pritchard et al. 2000, Falush et al. 2003), was used to determine hybrid identities as described by Xu et al. (2008). The Structure conditions used were the same as those used in Stewart et al. (2010). Structure yields *Q* values for each individual. Each *Q* value is an approximation of the likelihood that an individual belongs to one or more populations (or species). In this study, the *Q* values near one and zero indicate that individuals are loblolly pines or shortleaf pines, respectively. Hybrids were reported when predicted genome proportion levels were between of 0.953 and 0.047, these limits being about what is expected for trees in a third backcross generation and anything more closely related (Xu et al. 2008). Individuals were assigned to being F1s or hybrids backcrossed to loblolly up to 3 times ($Q = 0.375$ to 0.953), loblolly pine ($Q = 0.953$ to 1), shortleaf pine ($Q = 0$ to 0.047), or hybrids backcrossed into shortleaf pine up to 3 times ($Q = 0.047$ to 0.375). It is recognized that individuals may have a more complex genetic ancestry than these categories may indicate, but they are useful as a first approximation. Data representing loblolly pine and shortleaf pine trees from throughout the ranges of both species were included in the dataset to ensure that each calculated population referred to the two species, instead of some other population structure component. These data were taken from the study reported by Stewart et al. (2011).

To test whether the proportion of hybrids in the juvenile populations was different from the proportion of hybrids in the mature populations, a X^2 test was performed using the proportions of individuals that were assigned to each hybrid category: F_1s and loblolly pine backcrosses, shortleaf pine backcrosses, and shortleaf pine. The numbers of expected hybrid types in the juveniles were calculated using the frequency of these types observed in the mature populations. When calculating distances for the distance to the nearest plantation and the distances among populations, the approximate sample transect center was used to estimate distance from sample location to plantation location.

Results

In all sample sites except for the NE site, the hybridization rate of mature trees was greater than the hybridization rate of juvenile trees (Figure 2), but X^2 tests showed that any differences were not statistically significant. One mature population (NWP) had one F1 hybrid in it out of a sample of 24, as did its corresponding juvenile population (1 in 98). The juvenile population SW had two F1 hybrids out of 98 trees sampled. The SW population was the closest population to the loblolly pine plantations and had the most hybrids.

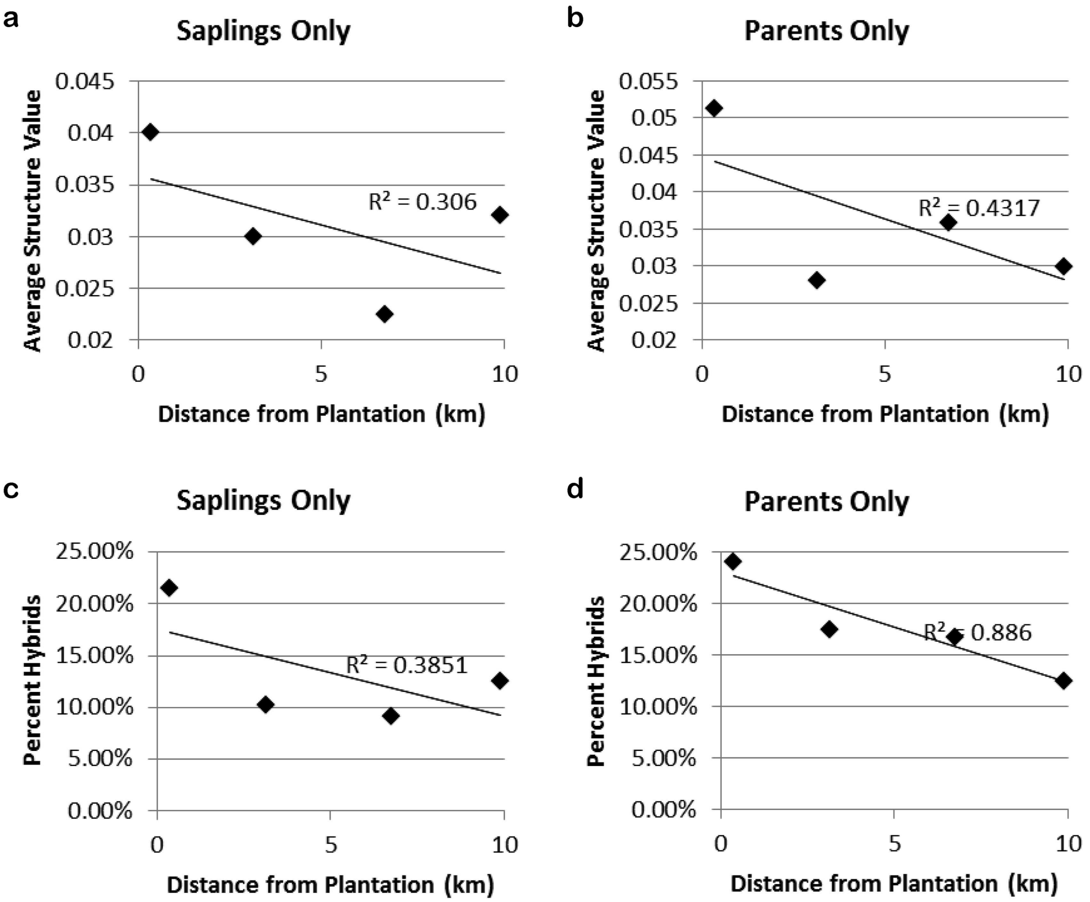

Figure 3. The correlation of distance from the loblolly plantations to the sample sites with the percent hybrids and the average Structure results. The correlation between the average Structure value of each study site and the distance to the nearest point in the loblolly pine plantations is shown for the juvenile (a) and the parents (b). Structure values of 0 indicate trees that are entirely shortleaf pine, and values of 1 indicate trees that are entirely loblolly pine. Values in between indicate different levels of hybridization. The averages were calculated as a mean of all values in a population. Hybrid percentages (the proportion of hybrids of any type) in the juvenile populations (c) and the parents (d) correlated with the distance from the nearest point of the loblolly pine plantations.

Most correlations between different measures of hybridity and geographic distance to the nearest loblolly pine plantations were nonsignificant ($P < 0.05$). However, all trend line slopes were negative (Figure 3). For example, the correlation coefficients (r) between the distance from the nearest loblolly pine plantation versus average Q value among the juvenile was -0.553 ($P = 0.223$). The correlation coefficient for the mature samples was -0.657 ($P = 0.171$). The correlation coefficient of the relationship between the nearest loblolly pine plantations versus the average Q values among the combined populations was -0.574 ($P = 0.0685$). However, the correlation coefficients between distances from the nearest loblolly pine plantations and the percent hybrids in each population tended to be significant: -0.621 ($P = 0.190$) among the juveniles, -0.941 ($P = 0.029$) among the mature trees, and -0.691 ($P = 0.029$) among the combined populations (Figure 3).

On average, the pines in this study had 2.50 needles per fascicle with an SD of 0.336, which is consistent with the common description of shortleaf pine having two or three needles per fascicle. The average needles per fascicle for each individual showed linear correlation with those individuals' structure values was $r = 0.106$ with $P = 0.010$.

Discussion

Hybridization and Introgression

The differences in hybridization and introgression among the populations in both time and space suggest that both factors have likely played roles in the causation of interspecies crossing. While none of correlations of offspring population hybridization with distance from loblolly pine plantations were statistically significant, there were significant correlations between the percent hybrids and distance from the loblolly pine plantations in both the parent populations and the combined offspring and parent populations. If more sites had been sampled, it is probable that the correlations of juvenile hybridization with distance from loblolly pine plantations would have been statistically significant as well. We believe that despite the variable statistical significance, there is meaningful biological significance.

An inverse relationship trend between the distance of the stands from the loblolly pine plantations and the levels of hybridization and introgression is expected. Stands that are nearer to the plantations should receive a denser cloud of viable loblolly pine pollen in the spring. Pine pollen is capable of long-distance flight (Williams 2010). Also, despite its small size of about 50 μm (Williams 2008), loblolly pine pollen is highly resistant to desiccation and ultraviolet light (Bohrervora et al. 2008). The distances observed in this study are short relative to the potential distance that loblolly pine pollen may travel.

Plant hybridization is dependent on the timing of pollination, since the species involved must occasionally shed pollen and be receptive to pollen to hybridize. Shortleaf pine and loblolly pine are receptive to and shed pollen at different times during the spring. Shortleaf pine is fertile between late March and late April (Lawson 1990), and loblolly pine is fertile between February and April (Schultz 1997). At any given location, the timing separation is usually about 2 weeks, with shortleaf pine succeeding loblolly pine. In both species, pollen is shed earlier in the south than in the north, and the northward position of shortleaf pine's range relative to that of loblolly pine in this study may account for the two species not hybridizing more often. Currently, there are no data available for the pollen shedding and receptivity times of any loblolly pine x shortleaf pine hybrids, but it may be fair to assume that the timing would be intermediate to the parent species. Studies have shown that hybrids between the two species are more common west of the Mississippi River than east of it, probably because higher climatic variability allows for more overlap in pollen shed and receptivity times (Edward and Hamrick 1995, Xu et al. 2008, Stewart et al. 2010). Since the sampling site in our study is west of the Mississippi River, hybridization is expected to be more common.

The shortleaf pine parental populations generally had about the same level of hybridization and introgression as their offspring did, which contrasts with the results in Stewart et al. (2011), where hybridization and introgression were much higher in recently collected tissue samples than they were in trees sampled from 50 years before. This discrepancy may be due to local dynamics. While trends in hybridization and introgression between these two species are regional, the real phenomenon occurs on the small scale, and each forested area is subject to local effects. Recent weather patterns may have prevented a large influx of hybrids, as the local rate of hybridization is widely assumed to be a result of weather variation. However, no studies have yet determined the effect of weather on hybridization, though Edwards and Hamrick (1995) suggested that the drier climate of the western ranges of the two species may contribute the higher rate of hybridization found there. The history of the site and the nature of the hybrids suggest that the parental rate of hybridization relative to the offspring rate might not be unexpected. The land south and upwind of the Caney Creek Wilderness lies in the Athens Piedmont Plateau, and pine stands there were initially managed by Dierks Forest Industries until 1969, when Weyerhaeuser Company purchased the land. Dierks used natural regeneration and uneven-aged silvicultural systems in their pine stands, and on the Athens Piedmont Plateau this system maintained uneven-aged stands dominated by loblolly pine (which company foresters favored) with a minor and varying proportion of shortleaf pine (Smith 1986). When Weyerhaeuser acquired the Dierks lands, they started a program of even-aged plantation management of loblolly pine (Smith 1986) that included planting improved North Carolina planting stock (Lambeth et al. 1984)

This history may explain the unchanged level of hybridization in the younger trees in the Caney Creek Wilderness through two counteracting trends. First, the mixed shortleaf pine-loblolly pine forests in the Athens Piedmont Plateau likely had many hybrids and backcrossed hybrids that may have shed pollen at times more concurrent with shortleaf pine than loblolly pine would have. These were replaced with pure loblolly pine, primarily of North Carolina origin (Lambeth et al. 1984). This change in pollen source for the Caney Creek Wilderness may have reduced the number of hybrids. Second, shortleaf pine x loblolly pine hybrids appear to combine the faster growth rate of loblolly pine and the drought tolerance of shortleaf pine. Without fire to control the hybrids, selection might favor the hybrids over shortleaf pines (Lilly et al. 2012), something that may slowly change proportion of hybrids in the juvenile population. Regular fire in a site like the Caney Creek Wilderness could have selected for nonhybrid shortleaf pines over time. There are no historic fire occurrence data specifically for the Caney Creek area, but fire scar analysis in nearby shortleaf pine-dominated forests suggests a return interval of about 7 years in the century prior to European colonization (Johnson and Schnell 1985). We suspect this would not be sufficiently frequent to give advantage one way or the other.

The shortleaf pine populations of the Caney Creek Wilderness Area appear to have a stable rate of introgression over the approximately two generations that were tested. More introgression was observed in sites nearer to the loblolly pine plantations, so it is likely that pollen from the plantations is contributing to the genetic makeup of the Caney Creek Wilderness Area shortleaf pines. These data suggest that even a limited distance of a mile or so is somewhat effective in limiting interspecific crossing between loblolly pine and shortleaf pine. However, it is also apparent that without changes to selection pressures (i.e., inclusion of fire and management in spatial positioning), it seems unlikely that most shortleaf pine populations can avoid introgression with loblolly pine over time.

Between 1953 and 1997, shortleaf pine acreage in the southeast United States decreased from 7.8 million acres to 4.7 million acres, while loblolly pine acreage increased from 35.6 million acres to 39.1 million acres (South and Buckner 2003). This relative coverage change increases the distribution and amount of loblolly pine pollen while decreasing by almost 50% that of shortleaf pine, which may increase the likelihood of hybridization with naturally regenerating shortleaf pine. When the management objective is to maintain or restore native shortleaf pine ecosystems, this imperils the genetic integrity of shortleaf pine as a species. Managers may consider planting shortleaf pine some distance away from loblolly pine plantations, but in light of the results here, sites that already have hybrid character will not necessarily perpetuate increasing introgression.

Management Implications and Recommendations

The data presented here suggest guidance for managers interested in maintaining pure shortleaf pine stands rather than hybrids. First, sites closest to loblolly pine pollen sources are more likely to have hybrids, so a buffer of other forest trees may decrease the rate of hybridization. The national forest in the Ouachita Mountains is one of the least parcelized in the region, but recent land exchanges have brought some industry loblolly pine plantations into federal ownership. To reduce hybridization, priority should be given to converting these loblolly plantations back to shortleaf pine, especially if they are small in area and in the middle of the federal ownership. Second, sites with existing hybrid character may not necessarily show increases in hybridization from generation to generation, at least in terms of seeds germinated. Using prescribed fire early in the establishment of new age cohorts obtained using the seed tree or shelterwood methods may selectively remove hybrids early. Thirdly, trained field personnel can quickly distinguish the most first-generation hybrids based on needle and cone morphology. This could be used to develop or modify silvicultural treatments for release, precommercial thinning, and early commercial thinning so as to mark the hybrids for removal. Given the lack of a functional basal crook of hybrid juveniles (Lilly 2011), prescribed fire might be an effective management tool to reduce hybrid survival and establishment. That opens the possibility that when planting shortleaf pine, planting stock should be carefully inspected, and juveniles should be culled if a prominent crook is not readily apparent.

Endnote

1. The supplementary table found at www.biomedcentral.com/content/supplementary/1471-2156-12-17-s1.txt describes all markers, except PtTX4205, which can be at www.ncbi.nlm.nih.gov/nuccore/21280621 (GenBank AF455076.1).

Literature Cited

Baker, J.B., and O.G. Langdon. 1990. *Pinus taeda* L. loblolly pine. P. 497–512 in *Silvics of North America. Volume 1. Conifers*, Burns, R.M., and B.H. Honkala (tech. coords.). USDA For. Serv., Agricultural Handbook 654, Washington, DC.

Chagne, D., P. Chaumeil, A. Ramboer, C. Collada, A. Guevara, M.T. Cervera, G.G. Vendramin, et al. 2004. Cross-species transferability and mapping of genomic and cDNA SSRs in pines. *Theory Appl. Gen.* 109:1204–1214.

Cotton, M.H., R.R. Hicks Jr., and R.H. Flake. 1975. Morphological variability among loblolly and shortleaf pines of east Texas with reference to natural hybridization. *Castanea* 40: 309–319.

Deemer, D.L., and C.D. Nelson. 2010. Standardized SSR allele naming and binding among projects. *Biotechniques* 49:835–836.

Echt, C.S., S. Saha, K.V. Krutovsky, K. Wimalanathan, J.E. Erpelding, C. Liang, and C.D. Nelson. 2011. An annotated genetic map of loblolly pine based on microsatellite and cDNA markers. *BMC Genet.* 12:17.

Edwards, M.A., and J.L. Hamrick. 1995. Genetic variation in shortleaf pine, *Pinus echinata* Mill. (Pinaceae). *For. Genet.* 2:21–28.

Edwards-Burke, M.A., J.L. Hamrick, and R.A. Price. 1997. Frequency and direction of hybridization in sympatric populations of *Pinus taeda* (Mill) and *P. echinata* (Pinaceae). *Am J. Bot.* 84:879–886.

Falush, D., M. Stephens, and J.K. Pritchard. 2003. Inference of population structure: Extensions to linked loci and correlated allele frequencies. *Genetics* 164:1567–1587.

Guichoux, E., L. Lagache, S. Wagner, P. Chaumeil, P. Léger, O. Lepais, C. Lepoittevin, et al. 2011. Current trends in microsatellite genotyping. *Mol. Ecol. Resour.* 11:591–611.

Hare, R.C, and G.L. Switzer. 1969. Introgression with shortleaf pine may explain rust resistance in western loblolly pine. USDA For. Serv., Res Note SO-88. 2 p.

Hicks, R.R. Jr. 1973. Evaluation of morphological characters for use in identifying loblolly pine, shortleaf pine, and loblolly x shortleaf hybrids. *Castanea* 38:182–189.

Huneycutt, M., and G. Askew. 1989. Electrophoretic identification of loblolly pine-shortleaf pine hybrids. *Silvae Genet.* 38:3–4.

Johnson, F.L., and G.D. Schnell. 1985. Wildland fire history and the effects of fire on vegetative communities at Hot Springs National Park, Arkansas. Biological survey, University of Oklahoma, Norman, OK. 44 p.

Lambeth, C.C., P.M. Dougherty, W.T. Gladstone, R.B. McCullough, and O.O. Wells. 1984. Large-scale planting of North Carolina loblolly pine in Arkansas and Oklahoma-a case of gain versus risk. *J. For.* 82:736–741.

Lawson, E.R. 1990. *Pinus echinata* Mill., shortleaf pine. P. 316–326 in *Silvics of North America. Volume 1: Conifers*, Burns, R.M., and B.H. Honkala (tech. coords.). USDA For. Serv., Washington, DC. 675 p.

Lilly, C.G., R.E. Will, and C.G. Tauer. 2012. Physiological and morphological attributes of shortleaf x loblolly pine F1 hybrid seedlings: is there an advantage to being a hybrid*? Can. J. Forest Res.* 42:238–246.

Mergen, F., G.R. Stairs, and E.B. Snyder. 1965. Natural and controlled loblolly x shortleaf pine hybrids in Mississippi. *For. Sci.* 11:306–314.

Nelson, C.D., S. Josserand, C.S. Echt, and J. Koppelman. 2007. Loblolly pine SSR markers for shortleaf pine genetics. P. 95–98 in *Shortleaf pine restoration and ecology in the Ozarks: Proceedings of a symposium,* Kabrick, J.M., D.C. Dey, and D. Gwaze (eds.). Tech. Rep. NRS-P-15.

Pritchard, J.K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. *Genetics* 155: 945–959.

Raja, R.G., C.G. Tauer, R.F. Wittwer, and Y.H. Huang. 1997. Isoenzyme variation and genetic structure in natural populations of shortleaf pine (*Pinus echinata*). *Can. J. Forest Res.* 27:740–749.

Schultz, R.P. 1997. Loblolly Pine-The Ecology and Culture of Loblolly Pine (Pinus taeda L.). USDA For. Serv., Agricultural Handbook 713, Washington, DC. 492 p.

Smith, K.L. 1986. *Sawmill: The Story of Cutting the Last Great Virgin Forest East of the Rockies.* University of Arkansas Press, Fayetteville, AR. 260 p.

South, D.B., and E.R. Buckner. 2003. The decline of southern yellow pine timberland. *J. For.* 101:30–35.

Stewart, J.F., Y. Liu, C.G. Tauer, and C.D. Nelson. 2010. Microsatellite versus AFLP analyses of pre-management introgression levels in loblolly pine (*Pinus taeda* L.) and shortleaf pine (*Pinus echinata* Mill.) *Tree Genet. Genomes* 6:853–862.

Stewart, J.F., C.G. Tauer, and C.D. Nelson. 2011. Bidirectional introgression between loblolly pine (*Pinus taeda* L.) and shortleaf pine (*P. echinata* Mill.) has increased since the 1950s. *Tree Genet. Genomes* 8(4):725–735.

Waggoner, G.S. 1975. Eastern Deciduous Forest, Vol. 1: Southeastern Evergreen and Oak-Pine Region in Natural History Theme Studies No. 1, Report No. NPS 135. 206 p.

Williams, C.G. 2008. Aerobiology of *Pinus taeda* pollen clouds. *Can. J. For. Res.* 38:2177–2188.

Williams, C.G. 2010. Long-distance pine pollen still germinates after meso-scale dispersal. *Am. J. Bot.* 97:846–855.

Williston, H.L. 1974. Managing pines in the ice-storm belt. *J. For.* 72:580–582.

Xu, S., C.G. Tauer, and C.D. Nelson. 2008. Natural hybridization within seed sources of shortleaf pine (*Pinus echinata* Mill.) and loblolly pine (*Pinus taeda* L.) *Tree Genet. Genomes* 4:849–858.

Zobel, B.J. 1953. Are there natural loblolly-shortleaf pine hybrids? *J. For.* 51:494–495.