Genetic Diversity and Hybridization in Seed Sources of Shortleaf Pine and Loblolly Pine

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Shortleaf pine (*Pinus echinata* Mill.) and loblolly pine (*Pinus taeda* L) are widely distributed over the southeast United States, thus it is reasonable to assume they possess a large amount of genetic variation due to wide adaptation. Southwide Southern Pine Seed Source Studies (SSPSSS) of both species were established in the 1950s, and provided much early range wide information. Schultz (1997), summarizing the SSPSSS and other studies of loblolly pine, reported the species possesses considerable natural variation for many morphological traits, and that this variation is generally clinal, extending both north to south and east to west. Some, but not all studies reported differences between populations east and west of the Mississippi River. Few studies of natural variation in shortleaf pine have been reported. The SSPSSS results, summarized at age 10 by Wells and Wakeley (1970), showed no geographic patterns, although northern sources survived best in northern plantings, and southern sources grew faster until moved too far north. In a Mississippi SSPSSS planting Wells (1973) noted that the only consistent genetic difference between east and west populations of shortleaf pine was in time of growth initiation, with sources west of the Mississippi River initiating growth earlier. Tauer (1980) reported that at age 20 two Oklahoma SSPSSS shortleaf pine plantings showed a north-south growth trend, but no east-west trend.

Early studies of natural variation relied on morphological traits, but later studies utilized monoterpenes and isozymes and they generally confirmed the morphological data, showing north-south and east-west gradients. However, some questions remained. There appeared to be some east verses west of the Mississippi River differences in loblolly pine (Wells and Lambeth 1983, Wells and Wakeley 1970), which Florence and Rink (1979) and Wells and Wakeley (1970) attributed to the presence of the river itself, while Schmidtling et al. (1999) and Wells et al. (1991) attributed the differences to separate east and west glacial refugia during the Pleistocene. There were few differences noted for shortleaf pine populations east and west of the river (Edwards and Hamrick 1995, Raja et al.1997), except that the frequency of heterozygosity of the *IDH* (isocitrate dehydrogenase) locus was higher west of the river. Heterozygosity at this locus indicates the tree is a shortleaf X loblolly pine hybrid (Huneycutt and Askew 1989), and the hybrid frequency in some western populations appears to be quite high (15% according to Raja et al. 1997 and Chen et al. 2004).

Current forest management favors loblolly pine, and many acres of shortleaf pine have been replaced with improved loblolly pine. The USDA Forest Service is one of a few organizations which regenerate shortleaf pine, usually relying on natural regeneration. As a result, naturally regenerated shortleaf pine stands are becoming surrounded by more and more loblolly pine. Raja et al. (1998) and Chen et al. (2004) reported a level of about 15% hybridization between these two species in west-central Arkansas. The long term effect of such a high hybridization

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level on species integrity is unknown. The samples collected in this study (from SSPSSS plantings) are from seeds collected in 1951 and 1952, when man's influence due to management was minimal. Thus, this study estimates genetic variation found in natural populations of shortleaf pine and loblolly pine approximately 50 years ago, prior to intensive management. These data will later be used as reference level data for addressing questions concerning diversity and hybridization level changes between these pine species from the 1950s to the present.

MATERIALS AND METHODS

More recently available DNA based markers can detect difference not easily discriminated by morphological traits or isoenzyme markers. Amplified fragment length polymorphism (AFLP) markers are easy to use for studying population genetics of trees as their use requires no previous sequence knowledge, has good repeatability and can detect multiple loci. In this study, to describe genetic diversity in natural stands of shortleaf pine and loblolly pine, needle tissue samples were taken from 93 shortleaf pine and 112 loblolly pine trees from 22 seed sources of SSPSSS plantings in Oklahoma, Arkansas and Mississippi. AFLP markers were developed and used to estimate genetic diversity and hybridization levels in these pine species.

RESULTS AND DISCUSSION

The 22 seed sources were grouped into 16 populations according to seed source geographic and physiographic region for the genetic diversity study. Of 48 primer pairs screened, 17 produced 794 AFLPs in shortleaf pine and 21 produced 647 AFLPs in loblolly pine. Analysis of these AFLP data showed high genetic diversity in both species with most of the genetic diversity within populations, in agreement with earlier studies discussed above. Analysis also showed gene flow is high among populations in both species, which explains the finding of no correlation between population genetic distances and geographic distances. Genetic differences between the east and the west regions were minimal. These results confirm the earlier studies based on morphology and isoenzymes and reinforce the appropriateness of current breeding strategies for both species in that most genetic variation lies within populations.

For the hybridization study, the 48 primer pairs screened revealed 17 primer pairs which produced 96 AFLPs polymorphic across loblolly pine and shortleaf pine. The *IDH* marker identified two loblolly pine and two shortleaf pine hybrids in the trees sampled. Two additional shortleaf pine hybrids were found by combining the 96 AFLPs with the *IDH* marker using software NewHybrids version 1.1 beta. Hybridization frequency varied geographically, ranging from 25% in Missouri to 0% in other sources in this study. The hybridization level was higher in populations west of the Mississippi River than east of the river (7.7% west vs. 0.7% east). If we consider five additional trees identified as having an average 36% probability of being hybrids, the rate would be 14.0% west vs. 4.0% east in shortleaf pine, 4.5% west vs. 2.2% east in loblolly pine and 10.8% west vs. 2.1% east in all populations. These results suggest that the existence of hybrids or the potential for creation of hybrids should be considered in forest management decisions, particularly for seed collection natural regeneration of shortleaf pine.

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LITERATURE CITED

Chen JW, Tauer CG, Bai G, Huang Y, Payton ME, Holley AG (2004) Bidirectional introgression between *Pinus taeda* and *Pinus echinata*: Evidence from morphological and molecular data. Can. J. For. Res. 34: 2508-2516.

Edwards MA, Hamrick JL (1995) Genetic variation in shortleaf pine, *Pinus echinata* Mill. (Pinaceae). For. Genet. 2: 21-28.

Florence Z, Rink G (1979) Geographic patterns of allozymic variation in loblolly pine. In: Proceedings of the 15th Southern Forest Tree Improvement Conference, June 19-21, 1979, Starkville, MS, pp. 33-41.

Huneycutt M, Askew GR (1989) Electrophoretic identification of loblolly pine shortleaf pine hybrids. Silvae Genet. 38: 95-96.

Raja RG, Tauer CG, Wittwer RF, Huang YH (1998) Regeneration methods affect genetic variation and structure in Shortleaf Pine (*Pinus echinata* Mill.) For. Genet. 5: 171-178.

Raja RG, Tauer CG, Wittwer RF, Huang YH (1997) Isoenzyme variation and genetic structure in natural populations of shortleaf pine (*Pinus echinata*). Can. J. For. Res. 27: 740-749.

Schmidtling RC, Carroll E, LaFarge T (1999) Allozyme diversity of selected and natural loblolly pine populations. Silvae Genet. 48: 35-45.

Schultz RP (1997) Loblolly Pine: the ecology and culture of loblolly pine (Pinus taeda L). USDA, Ag. Handb. 713.

Tauer CG (1980) Twenty-year results of a shortleaf pine seed source study in Oklahoma. OSU Ag. Exp. Sta. Bull. B-752. pp12.

Wells OO (1973) Variation among shortleaf pines in a Mississippi seed source planting. USDA For. Serv. Res. Note 3-SO-162. pp8.

Wells OO, Lambeth CC (1983) Loblolly pine province test in southern Arkansas 25th year results. South. J. Appl. For. 2:71-75.

Wells OO, Switzer GL, Schmidtling RC (1991) Geographic variation in Mississippi loblolly pine and sweetgum. Silvae Genet. 40: 105-119.

Wells OO, Wakley PC (1970) Variation in shortleaf pine from several geographic sources. For. Sci. 16:415-423.