THE RATE OF HYBRIDIZATION AND INTROGRESSION BETWEEN LOBLOLLY PINE (*PINUS TAEDA* L.) AND SHORTLEAF PINE (*PINUS ECHINATA* MILL.) HAS INCREASED MARKEDLY SINCE THE 1950S.

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Loblolly pine (*Pinus taeda* L.) and shortleaf pine (*Pinus echinata* Mill.) are important forest species that have large ranges across the southeastern United States that share a large sympatric range in addition to their own allopatric ranges. The two species have been crossed artificially (Schreiner 1937), and natural hybrids have been observed (Hare and Switzer 1969). It is thought that hybridization is normally prevented by the different flowering time in the two species, but when the climatic conditions are right, hybridization may occur. Earlier studies used morphology and later isoenzymes to identify natural hybrids, but recent studies have used DNA markers to identify hybrids. Xu et al. (2008a, b) reported hybrids in study samples from material grown from seed collected in the 1950s from the Southwide Southern Pine Seed Source Study (SSPSSS) using amplified fragment length polymorphism (AFLP) markers. Stewart et al. (2010) followed up on that study using short sequence repeat (SSR) markers, also called microsatellite markers, to identify hybrids in the same source material.

In this study, we used microsatellites to characterize the hybrid status of trees collected from current stands from the same counties that were represented in Xu et al. (2008a, b) and Stewart et al. (2010). The goal of this study is to compare the rates of hybridization and introgression in modern stands to those from the 1950s. From the 1950s to present, the rates of hybridization and introgression in both species have increased dramatically. Introgression can be a major threat to species, even leading to extinction, and increased introgression in many species has been connected to human activities (Wolf et al. 2001).

Materials and Methods

Green leaves from both species were collected by foresters in the same counties as those collected for the studies by Xu et al. (2008a), Xu et al. (2008b), and Stewart et al. (2010) i.e., the SSPSSS. Loblolly pine samples were collected from 9 counties east of the Mississippi River and 2 counties west of the river, and shortleaf pine samples were collected from 6 counties east of the Mississippi River and 4 counties west of the river. DNA was extracted from the needles using the Qiagen DNEasy Plant Minikit (Qiagen, Valencia, CA.)

Twenty-five microsatellite markers previously confirmed to be polymorphic in both speices were used in this study. Three primers for these markers were used during PCR, two that flanked the short sequence repeat region and one primer labeled with a fluorophore. All PCR products were scored using a LI-COR 4300 DNA Analyzer (LI-COR Biosciences, Lincoln, NE).

Structure version 2.3.2 was used to determine hybrid character of individuals. We set population number k to 2, which represents the two species analyzed in this study. Hybrids were reported when predicted genome proportion levels (*Q*) were between 0.9531 and 0.0469, about what is expected for trees in an F1 cross or a first through third backcross generations.

Results and Discussion

The rates of hybridization and introgression increased markedly in both species: 27.3% hybrids in loblolly pine populations and 45.7% hybrids in shortleaf pine populations compared to rates of 4.5% and 3.3%, respectively, in the 1950s populations. West of the Mississippi River, the shortleaf pine hybridization rate increased from 7.5% to 54.0%, and the loblolly pine hybridization rate increased from 9.1% to 20.0%. East of the Mississippi River, the hybridization rate for shortleaf pine increased from 0% to 40.0%, and the hybridization rate of loblolly pine increased from 2.2% to 29.2%.

Introgression is a known cause of extinction of species—or, to be more precise, genomes (Allendorf et al. 2001). In general, hybridization can threaten a taxon in a wide variety of ways, through the generation of poorly adapted hybrids, the generation of hybrids with greater vigor than one or more of the contributing species, or the introgressive extinction of one or more species (Simberloff 1996). Discovering whether introgression is a natural process or anthropogenic is crucial to understanding how or whether to manage the issue (Allendorf 2001). Given the timescale for change in introgression in this study (about 50 years), it is almost certain that the cause is, at least in large part, human caused in this case.

Human causes for introgression include introduction of plants and animals, habitat fragmentation, and habitat modification (Allendorf & Luikart 2007). All three could have an impact on loblolly pine, shortleaf pine, and their hybrids. Loblolly pine is being planted outside of its range, as well as being planted as a replacement for lost/harvested shortleaf pine stands, and there is evidence that shortleaf pine genes have been introgressing into the allopatric loblolly pine populations. Habitat fragmentation is common in the southeastern United States, a factor that can lead to the mixing of previously distinct gene pools (Rhymer & Simberloff 1996). In the case of loblolly pine and shortleaf pine, habitat fragmentation could lead to more opportunities for cross-pollination. As both species are early successional pines, they will often invade the disturbed sites generated by human development, a process that can create a corridor for the two species to more often enter each other's habitat (Rhymer & Simberloff 1996). These corridors may change the frequency of contact and encourage introgression by becoming hybrid zones, or regions where two species often intercross to create hybrids (Wolf et al. 2001). One other important form of habitat modification for this case is the planting of loblolly pines in shortleaf pine habitats, often as replacement trees for lost/harvested shortleaf pine stands.

The ecology of loblolly pine and shortleaf pine is rapidly changing, as human activity and forest management make their marks on the distribution of these two species. It appears that hybridization and introgression are phenomena with increasing effects on both pine species, and the future of these two species is difficult to ascertain. Through habitat modification, global warming, fire suppression, seed/seedling movement through artificial regeneration, mankind is

altering the genetic makeup of loblolly pine and shortleaf pine. While it is beyond the scope of this study, management practices regarding these two species need to be reexamined to determine their ecological efficacy.

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