HYBRIDIZATION IN NATURALLY REGENERATED SHORTLEAF PINE NEAR ARTIFICIALLY REGENERATED STANDS OF LOBLOLLY PINE

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Shortleaf pine (*Pinus echinata* Mill.) is an important timber species found throughout the southeastern United States. The species is known to hybridize with loblolly pine (*Pinus taeda* L.), another very important timber species in the southeastern United States. 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. In the Mid-South, the natural range of loblolly pine ends at the northern edge of the upper west Gulf Coastal Plain; as a result, shortleaf pine is the only naturally-occurring pine in the Ouachita and Ozark Mountains, where it often forms pure pine-dominated stands especially on southern aspects. The transition from mixed loblolly pine and shortleaf pine stands to shortleaf pine stands without loblolly pine is occasionally patchy. Additionally, timber companies prefer to plant loblolly pine in their plantations, which may be outside of the natural range of the species.

Factors leading to introgression in loblolly pine and shortleaf pine may include the distance between stands, or the common condition in which both species are naturally found together in stands in the upper west Gulf region. In the present study we used microsatellites 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 allopatric by about 40 kilometers relative to loblolly pine. However, extensive plantations of loblolly pine have been established in this area over the past four decades on private lands managed intensively for timber and fiber productivity in support of local forest industry.

Materials and Methods

Current-year leaves were collected from four locations in the Caney Creek Wilderness Area, Polk County, Arkansas, which is part of the Ouachita National Forest. Each collection location was on the corner of an approximate rectangle about 32 km from east to west and 24 km from north to south. Collection sites were thus labeled northwest (NW), southwest (SW), northeast (NE), and southeast (SE). Twenty-five possible parent trees—trees that were at least 30 years old and labeled NWp, SWp, NEp, and SEp, depending on the source location—were sampled from each location, and 100 naturally regenerated seedlings/saplings, which were labeled NW, SW, NE, and SE, were also sampled from each location. The DNA was extracted from leaf tissue through cutting the leaves into small pieces and then using the Qiagen DNeasy 96 Plant Kit (Qiagen, Velencia, 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).

General population genetic analyses were performed with the software GenAlEx 6.3, which calculated Φ_{PT}, Nei's genetic distance, the inbreeding coefficient (F_{IS}), expected heterozygosity (H_E), observed heterozygosity (H_O), and Hardy-Weinberg Equilibrium statistics. 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. In order to test whether the proportion of hybrids in the sapling populations was different from the proportion of hybrids in the parent populations, a X^2 test was performed, using the proportions of individuals that were assigned to each hybrid category: F1s and loblolly pine backcrosses, shortleaf pine backcrosses, and shortleaf pines.

Results and Discussion

The estimate of population differentiation (Φ_{PT}) was calculated across all 8 populations to be 0.064, which is normal for a wind-pollinated forest tree and is in agreement with previous measures for the species (Stewart et al 2010). The correlation coefficient (R^2) for geographic distances and genetic distances of the populations was 0.103, which is insignificant (p= 0.057). Average expected heterozygosity (H_E) for all populations was 0.514. For the parent populations, average H_E was 0.497, and for the sapling populations, average H_E was 0.531. Average observed heterozygosity (H_O) for all populations was 0.422, while average H_O for the parent populations was 0.409, and average H_O for the sapling populations was 0.435. The mean inbreeding coefficient (F_{IS}) was 0.176, indicated little inbreeding. F_{IS} for the saplings (0.169) was similar to F_{IS} for the parents (0.189). Of the 25 markers used in this study, 5 of them passed the Hardy-Weinberg Equilibrium test, showing that the population as a whole is in transition.

In all sample sites, the measured number of hybrids decreased, but chi-square tests showed that any differences were not statistically significant. Correlations for different measures for relationship between the geographic distance to the nearest loblolly pine plantations and levels of hybridization were all insignificant. However, all trend lines were negative, which would be expected, as trees more distant from the plantations should have less hybridization than trees that are closer to the plantations. The relationship between the distance from the nearest loblolly pine plantation versus average *Q* value among the saplings was $R^2 = 0.3060$ ($p = 0.447$). That relationship among the parents was $R^2 = 0.4317$ ($p = 0.343$). The relationship between the nearest loblolly pine plantation versus the average *Q* value among the combined populations was $R^2 = 0.3293$ ($p = 0.137$). The relationships between distance from the nearest loblolly pine plantation and the percent hybrids in each population was $R^2 = 0.3851$ ($p = 0.379$) among the saplings, $R^2 = 0.886$ ($p = 0.059$) among the parents, and $R^2 = 0.4768$ ($p = 0.058$) among the combined populations. The lack of correlation may be due to an insufficient number of sites sampled and the sites being too close together.

On average, the pines in this study had 2.50 leaves per fascicle with a standard deviation of 0.336, which is consistent with the common description of shortleaf pine having two or three leaves per fascicle. The average leaves per fascicle for each individual did not correlate with those individuals' structure values, either when all individuals were included (R^2=0.01) or when only hybrid individuals were included (R^2=0.06).

References Cited

Stewart JF, Liu Y, Tauer CG, Nelson CD. 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.