

DNA MARKERS TO IDENTIFY SOUTHERN PINES AND THEIR HYBRIDS

Craig Echt¹, Sedley Josserand¹, Barbara Crane², Valerie Hipkins³, Chuck Burdine¹, C Dana Nelson¹, and James Barnett⁴

¹USDA Forest Service, Saucier, MS, USA

²USDA Forest Service, Atlanta, GA, USA

³USDA Forest Service, Placerville, CA, USA

⁴USDA Forest Service, Pineville, LA, USA

The issue of pine inter-species hybridization is a growing concern for National Forest System (NFS) seed orchards that provide seed for reforestation on NFS lands in the Southern Region. Because NFS seed orchards also sell surplus seed to state nurseries, which in turn can sell the seedlings to private landowners, hybridization is becoming a concern for these stakeholder groups. Climate change may be increasing chances for hybridization among pine species and there is speculation that some superior performing trees selected in the early days of Southern Region seed orchard establishment may be inter-species hybrids. Because the Forest Service has an obligation to make sure that its seed supply is genetically pure (not hybridized), the NFS Genetics program in conjunction with the National Forest Genetics Laboratory (NFGEL) will DNA fingerprint all longleaf and shortleaf pine trees and seed lots in NFS seed orchards. The goal is to remove seed producing trees that are inter-species hybrids and provide a quality check of current and future seed lots for the extent of species hybridization that may arise from off-site pollen sources. Ensuring species integrity of longleaf pine and shortleaf pine seed supplies will go a long way in Forest Service support of restoration initiatives for these species.

Hybrid seed producing trees in seed orchards and seasonal hybridization of longleaf and shortleaf seed trees by loblolly pine pollen are the two major practical concerns for orchard seed integrity. Because chloroplast DNA (CP) is only inherited through pollen in pines, we set out to develop easy-to-use diagnostic CP markers that would be unique to loblolly chloroplast genomes. While the main intent was to identify first-generation hybrids, this type of marker system obviously could also identify backcross hybrids that have a loblolly paternal lineage, though such higher generation hybrids, expected to be rare, are not an immediate concern for seed orchard operations. As part of a project with the Pennsylvania Bureau of Forestry, we also evaluated CP markers to identify hybrids between shortleaf and pitch pines. All markers were based on comparisons of pine CP sequences found in GenBank.

From a number of new CP markers evaluated, we found two that appeared species-specific based on our limited testing completed to date. One, in the tRNA gene for leucine, appeared as a single allele (gene fragment) only in loblolly pine ($n = 49$) and was not found in longleaf ($n = 133$), shortleaf ($n = 153$) or pitch pines ($n = 8$). The other, in the *ycf1* gene of unknown function, provided what appeared as species-specific size alleles: three alleles in loblolly ($n = 27$), four in longleaf ($n = 82$), one in shortleaf ($n = 142$), and two in pitch pine ($n = 6$). While both markers can discern loblolly hybrids, the later marker can also identify other types of species hybrids. The two CP markers together have identified a longleaf x loblolly hybrid seedling from a small sample of a longleaf pine seed lot, confirmed three known pitch x loblolly trees, and possibly identified several shortleaf x pitch and pitch x shortleaf hybrids from Pennsylvania. Additional sampling is underway for each species, plus slash pine, to estimate the extent of each marker's species-specificity. Even if not strictly species-specific, as long as the frequency of shared alleles is sufficiently low, these markers could still be used to estimate proportions of hybridization in seed lots and identify orchard trees that are likely hybrid.

Contact Information: Craig Echt, USDA Forest Service, 23332 Success Rd, Saucier, MS 39574, Phone: 228-832-2747 x210, Email: cecht@fs.fed.us