## A148 Using *Pinus* STR Profiling to Discriminate Pollen Samples at the Regional Level: A Potential Tool for Forensic Investigations

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After attending this presentation, attendees will understand the potential to use Short Tandem Repeat (STR) markers to perform DNA profiling of pollen samples from *Pinus echinata*.

This presentation will impact the forensic science community by providing another quantifiable piece of evidence that investigators can utilize to solve crimes and will demonstrate the potential to use short tandem repeat (STR) markers to perform DNA profiling of pollen samples from *Pinus echinata*. The use of a DNA-based STR analysis investigation using primers designed for previously identified STR markers in *P. echinata*, located regionally in southeast Texas for the study of localized population dynamics, may enable discrimination of pollens at regional and local levels. Eventually, this would allow an association between hortleaf pine tree populations or individual pines and associated pine pollen during a forensic investigation.

Advances in plant genomics have had an impact in the field of forensic botany. However, the use of pollen DNA profiling in forensic investigations has not yet been applied. DNA profiling of *P. echinata* pollen using five short tandem repeat (STR) markers is described herein and data supports that there is sufficient genetic variation to discriminate pollen samples at the regional level in southern Texas. Genomic DNA was extracted from pollen and needle samples and then quantified by real time PCR. Species were verified by sequencing the internal transcribed spacer (ITS) regions. DNA was amplified by PCR using fluorescent dyes and the genotype analysis by capillary electrophoresis revealed distinct DNA profiles from trees from 10 selected geographical sites.

This study has demonstrated that a preliminary multiplex STR system, developed from already existing STR markers for *P. taeda* for the study of pine population dynamics, may potentially be used for forensic identification purposes in other pine species. Even though PCR primers were not designed for the particular species of interest, they are sufficiently homologous to enable screening of polymorphic sites as a proof-of-principle for marker evaluation. The use of *P. taeda* primers for the amplification of STRs in the closely related species *P. echinata* was demonstrated to discriminate pollen samples from ten different sites. A DNA extraction method for pollen and a real-time PCR method for accurate pine DNA quantitation were used. In addition, five STR loci from both pollen and needle DNA were successfully typed. DNA profiles obtained from pollen material (*P. echinata*) at ten different sites were perfectly differentiated. When pollen DNA profiles were compared to their conifer donors (reference), no evidence of mixtures was detected for the five amplified STR loci. A population survey indicated the cumulative probability of identity using these five loci could be as low as one in 189. However, a probability to the pollen needle match was not assigned since two out of five markers utilized in this study showed departures from Hardy-Weinberg equilibrium. The causes of Hardy-Weinberg disequilibrium may be due to primer mismatches, small sample size, or the effects of population substructure. Further research is needed to develop a valid statistical approach and a reference database for associations or matches. Despite the departure of markers from Hardy-Weinberg equilibrium, our results strongly suggest that the use of genetic profiling in forensic botany has the potential to offer invaluable evidence in solving crimes where pollen is properly identified, packaged, stored, and analyzed.

**Forensic Science, DNA Typing, Forensic Botany**

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