B150 Modern Single Grain Forensic Palynology: Preserving the Evidence for a Comprehensive Analysis

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Learning Overview: After attending this presentation, attendees will have gained an understanding of a non-destructive genomic analysis application of single pollen grains by combining the techniques of quantitative Polymerase Chain Reaction (qPCR), DNA sequencing, and microscopy.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing a simplified and quantitative pollen grain analysis by combining two commonly practiced protocols that can be utilized in forensic labs at the state or local levels.

Palynology, the study of pollen grains, is known to play an essential role in forensic casework. Forensic palynology uses pollen as evidence to link an object or person to a particular time or place and has played a crucial role in many criminal investigations, from burglary to homicide, rape, war crimes, terrorism, and drug trafficking.

The identification of plant species from their pollen relies on two traditional methods: microscopy or genetic analysis. On the one hand, microscopy relies on identifying pollen grains through morphology (i.e., size, shape, and wall structure) and comparing it to an image library for accurate identification. On the other hand, genetic analysis characterizes pollen species using a short DNA sequence from a universal standard in the genome. Both methods have so far been mutually exclusive. The standard procedure for microscopy is to clean the grain through acetolysis, which destroys any genetic material in the sample. Studies involving genomic characterization of the plant material requires the release of the genomic material by mechanically crushing the grain, which can no longer be analyzed for morphology through microscopic methods. While the number of forensic palynological studies increases, they usually rely on only one of the two techniques above and rarely show the potential for an efficient analysis of individual grains within an assemblage to statistically evaluate the species distribution in the mixture of grains that can be the evidence.

This study considers that for forensic analysis, a non-destructive method for such evidence at the single grain level is essential. Keeping the evidence intact after DNA analysis allows secondary analysis in the future by microscopy if needed. Furthermore, the ability to perform genomic analysis for each pollen grain of the evidence bypasses the issue of DNA mixtures more likely to be observed in a pollen assemblage. To respond to this need, a new method was developed that does not destroy the pollen grain and allows for the extraction of genetic material on a single grain level.

In this study, *Pinus echinata* (shortleaf pine), *Taxodium distichum* (bald cypress), and *Plantago lanceolata* (ribwort plantain) individual pollen grains were used to demonstrate this approach, with qPCR of the chloroplast genetic marker rbcLw and the second nuclear ribosomal ITS2 region, DNA sequencing, and digital microscopy, confirming the non-destruction of the pollen grains. The DNA obtained non-destructively from single grains opens the door for other downstream applications, including plant species identification at the individual grain level in samples such as soil and clothing swabs.

DNA, Pollen, Palynology