B69 A Non-Destructive Genomic Analysis of Single Pollen Grains

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Learning Overview: After attending this presentation, attendees will have gained an appreciation of a non-destructive genomic methodology of single pollen grains that can be complementary to their microscopic analysis.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing a simplified, non-destructive procedure for the analysis of every single pollen grain in a sample, which can be utilized in forensic labs at the state or local levels.

With more than 300,000 plant species on the planet, palynology (i.e., the study of the structure and origin of pollen) plays an important role in forensic analysis. Each location has a unique flora at different periods in time, making pollen a perfect proxy to link person(s) or object(s) to a particular place or time. This is the basis of forensic palynology. It relies on the facts that: pollen is an ever-present feature of the environment; different geolocations have different pollen signatures, allowing for inference related to spatial tracking; and plants bloom at different times, allowing for temporal inference. Pollen is extremely durable and can be used for forensic studies decades after sample collection.

Forensic palynology has played a role in a large number of criminal investigations worldwide, including homicide, violent assault, rape, genocide, terrorism, suspected terrorism, and even intelligence on drug smuggling.

The considerable number of potential plant species that would need identification is handicapped by the few ones that are databased using traditional methods, reducing the potential for geolocation. These traditional methods rely on the examiner having a high level of expertise in microscopy and can be time-consuming. With the emergence of DNA barcoding, pollen genomics has an immense potential to classify and identify pollen taxonomically from its genetic signature. This approach characterizes pollen species using a short DNA sequence from a universal standard in the genome and can help transform the current standard vision of forensic palynology by making it readily accessible to a wide range of forensic laboratories and increase the taxonomic resolution of identification. In plants, three regions of the chloroplast genome (matK, rbcL, and trnH-psbA), as well as the nuclear ribosomal ITS2 region have been widely accepted for use as DNA markers, either in combination or separately. Two main advantages of using DNA barcoding in forensics are that: (1) it allows taxonomic identification of parts of the organism that do not display diagnostic morphological characters; and (2) the same method is used across multiple taxonomic groups, so it allows species to be classified without having to involve narrowly defined taxonomic experts.

To date, the most common cleaning process is acetolysis, which involves highly acidic conditions and chemicals that could damage or alter the exine morphology, in addition to destroying the pollen genetic material. Similarly, DNA analysis relies traditionally on the mechanical destruction of the pollen grain to liberate the genetic material. In this work, it is shown that single pollen grain can be examined using universally accepted genetic markers (rbcL, matK, and ITS2) for DNA barcoding using quantitative Polymerase Chain Reaction (qPCR) while keeping their morphological features for microscopy. This is possible by a simple low-temperature treatment of the pollen grain in ethanol, scalable from large quantities down to a single grain. For the first time, DNA and morphological information can be obtained for every single pollen grain in a sample, which can be stored for future examination.

DNA, Pollen, Palynology

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