Pollen Analysis: An Underutilized Discipline in the U.S. Forensic Science Community

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Introduction

Forensic palynology is the application of palynomorph analysis to the law. Palynomorph assemblages include modern and fossil pollen, spores, and other acid-resistant plant materials (i.e., some algae, fungal spores) that are abundant, easily preserved, and closely correlated with local environments. The discipline of forensic palynology typically encompasses the examination of pollen and spores recovered from people, objects, and locations associated with a crime. The Trace Evidence Unit of the FBI Laboratory is assessing the use of pollen analysis to augment soil and mineralogical examinations that are currently performed. These complementary analyses support a multi-disciplinary approach to trace evidence examinations in casework.

This paper provides forensic examiners, law enforcement personnel, and legal professionals a review of pollen analysis, its potential use in forensic casework, and the factors that have limited its application in the United States. We outline protocols to conduct pollen analysis, including evidence and sample collection, processing options, issues with identification and interpretation, and potential advances. Finally, a case study is presented from a kidnaping that included soil and pollen analysis conducted by the FBI and US Geological Survey (USGS).

Pollen and Forensics

Pollen and spores are ideal forensic trace materials because the grains are small, highly variable, and found on almost any item that has been exposed to or comes in contact with the air (Milne et al. 2005). Transfers can occur between people, plants, and objects or a combination of contact between these things. Their ubiquity in the environment is due to the prolific production of pollen by plants. The walls of pollen grains are composed of compounds that are highly resistant to most forms of decay enabling recovery from a range of evidentiary items and reference samples. The wide variety of shapes, sizes, and surface characteristics of pollen grains aid their identification (Figure 1).

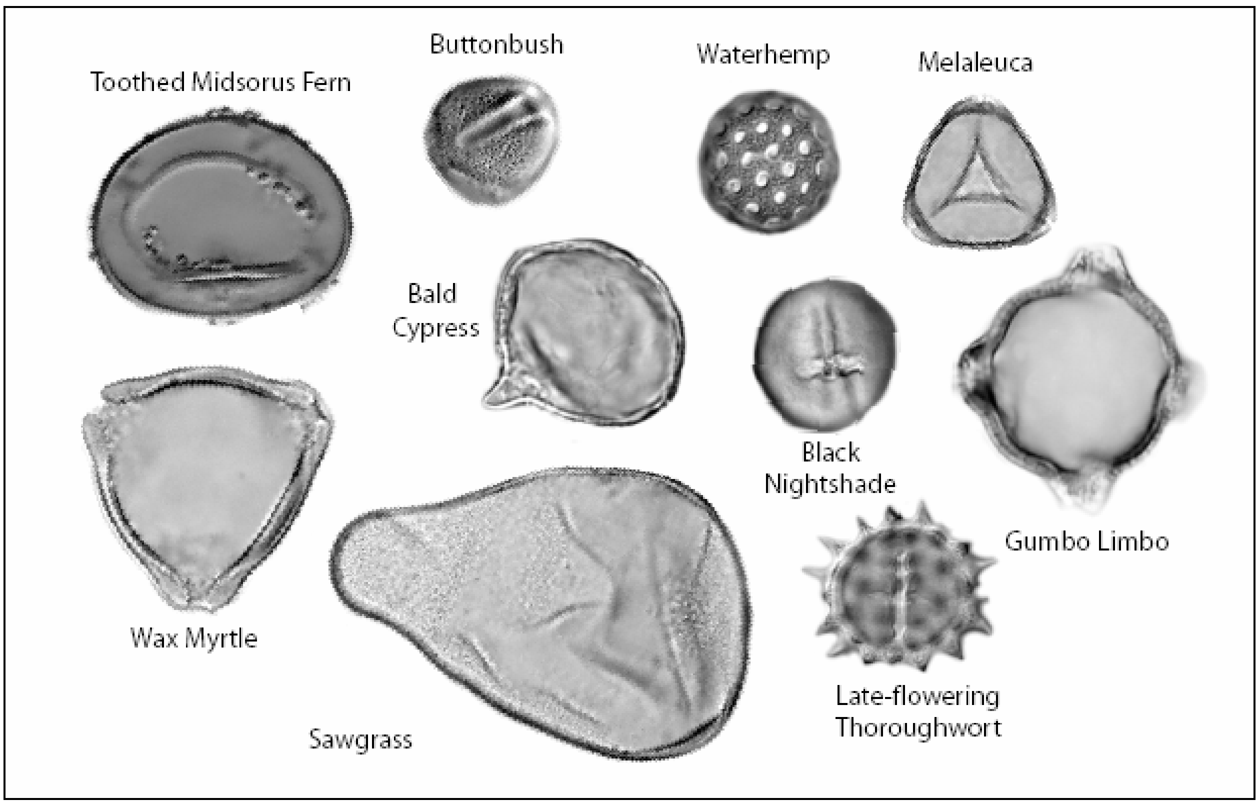

Figure 1. Examples of the variations in palynomorph morphology. These examples are from South Florida (Willard et al. 2004).

All of these characteristics can be exploited for a variety of scenarios common in forensic science—comparison of items to assess a common origin, evaluating alibis, assisting investigations by providing lead value, and attempting to identify the source of a sample through provenance studies.

Pollen and Evidence

Pollen can be isolated from most items typically submitted for forensic examination. These include soil, clothing and fabrics, ropes and twines, air filters, drugs, plant material, and human and animal material, such as hair, fur, and stomach contents (Horrocks 2004; Milne et al. 2005). In addition, samples for comparison purposes (i.e., alibi and exclusionary samples) and plant samples for use as reference material should be collected. The FBI Mineralogy group of the Trace Evidence Unit suggests soil reference samples be collected from the location and depth of interest including samples with any observable changes in color, texture, or composition. Furthermore, at least one additional sample set should be collected from at least 50 to 100 feet away from the location of interest. If pollen analysis is desired, local vegetation associated with a crime scene or alibi location should be collected for comparison purposes as well.

Pollen Recovery

Specific methods for recovering pollen from items are described in numerous references (Eyring 1996; Horrocks 2004; Traverse 1988). A general summary of the techniques includes the removal of mineral and organic matter through a series of acid treatments, sieving to consolidate the size fraction of interest, staining for better visibility and identification, and mounting the remaining pollen-rich residue on microscope slides for microscopic analysis. Processing protocols in the USGS Pollen Laboratory follow standard palynological techniques outlined in Traverse (1988). However, deviations are appropriate based on they type of material submitted for processing. Often, palynologists assess items for processing and apply the most appropriate approach for recovery.

Although pollen processing and identification are best conducted by an experienced palynologist, the preparation techniques used to process and recover pollen can be conducted at forensic laboratories with only a few minor adaptations. For the non-pollen specialist, contamination is the greatest issue, and it should be mitigated at every stage of the analysis, beginning with collection of samples in the field and continuing through every processing step in the laboratory. Suggestions for limiting or eliminating contamination include the use of clean, disposable collection equipment and processing supplies and "clean" rooms and practices for laboratory processing. These suggestions are detailed in Horrocks (2004) and Milne et al. (2005). Most of the suggested supplies and recommendations are easily obtained in forensic science laboratories.

Pollen as Evidence

The type and abundance of pollen present in a sample, also referred to as the pollen assemblage, is directly related to the plant taxa present at and near a given site. Most pollen in terrestrial sites originates from local and regional wind-pollinated plants, and the relative abundance of each species in an assemblage may be unique for a given location because different ecological communities produce different assemblages (Milne et al. 2005). However, several factors affect the probative value of pollen evidence (Milne et al. 2005). These include assessing if the appropriate questions are being asked, correctly identifying the pollen and plant types present at the crime scene, knowing the production and dispersal patterns of each taxon present as well as the spatial distribution of a particular type of pollen assemblage, and assessing the significance of an association between pollen assemblages. For example, pollen assemblages from different regions of the continent are clearly distinguishable, but the ability to narrow down the source area further depends on the presence of pollen produced by plants with a restricted distribution.

Current Status

Although routinely utilized in forensic case work in a growing number of countries, pollen analysis has seen extremely limited use in the United States forensic science community. Over the last three decades, law enforcement personnel in Great Britain, Australia, and New Zealand have come to appreciate the full potential of pollen analysis in their investigations. This is due to the efforts of dedicated practitioners using a combined approach of educating the law enforcement community and demonstrating the impact pollen analysis can make through its application in a wide range of cases (Bryant and Jones 2006; Milne et al. 2005). However, despite the efforts of practitioners in the United States to publicize palynology's potential in casework, most forensic palynology is still conducted by a handful of academics and consultants. Although these researchers have gone to great lengths to advertise the usefulness of pollen in forensic casework, its potential remains poorly realized in the law-enforcement community.

This limited use can be attributed to several factors that make this discipline inaccessible to forensic examiners and laboratories. These include a general lack of awareness of the science within the law enforcement community, the lack of trained palynologists in the forensic science community, and the specialized training necessary for pollen processing, identification, and interpretation. In addition, several issues exist from the perspective of the experienced palynologist. Most academic or research scientists have limited to no experience in the legal setting in which they find themselves when testifying in court proceedings. Inexperience (or limited previous experiences) with the proceedings of a trial may lead to an unwillingness of experienced palynologists to testify in court (Bryant and Jones 2006; Milne et al. 2005).

Steps Forward

Forensic palynology should become more accepted through increased awareness and accessibility within the forensic science, law enforcement, and legal communities. This includes educating members of these communities to the full potential of pollen analysis in casework. Conversely, the forensic science community must reach out to pollen specialists in order to fulfill the potential. Even if forensic laboratories and examiners do not possess the specialized training or experience to conduct pollen analysis, the following steps can be taken to make forensic palynology more accessible for use in casework.

Establishing and incorporating proper procedures for collecting and processing items for pollen will ensure this technique does not get overlooked. Internally, laboratories can establish or incorporate protocols for pollen analysis into their operating procedures. Externally, laboratories can educate and work with responders to ensure they are aware of collection and contamination issues.

Fostering relationships with regional universities and local, state, or federal agencies that conduct pollen analysis will make outside assistance more accessible to the forensic science community. For example, universities with botany and agriculture programs are a valuable resource for information on regional vegetation and plant reference materials. A relationship with a university, in turn, can expose academic programs and students to the forensic applications of science disciples.

Pollen data also must be interpreted and placed in an appropriate context for evaluation by forensic scientists, law enforcement officials, and, ultimately, the legal community. Datasets containing modern pollen assemblage and distribution information have been compiled by academic and government institutions conducting environmental research and allergy studies. These datasets cover local, state, national, or even worldwide pollen distributions. The Global Pollen database administered through the National Climate Data Center (NCDC) serves as a repository for data generated from environmental studies conducted around the world, including Africa, the Americas, and northern Asia. Particularly useful is the North American Pollen Database (NAPD), which includes pollen datasets and associated metadata from modern samples of surface soils and fossil sediments.

Although these datasets are valuable resources, they are not designed for easy navigation by the non-specialist, thus limiting their usefulness for the forensic community. This could be remedied by consolidating existing data that is of use to law enforcement investigations into a more accessible format and user-friendly platform. A particularly useful compilation would integrate data on modern pollen abundance and distribution with data on plant distribution, mineral associations, soil characteristics, and other parameters. This would include existing surface sample and reference data, and would integrate new data acquired through casework and related research as it is generated.

Case Example: New Hampshire vs. Manuel Gehring

This case example illustrates how pollen analysis can greatly enhance the impact of trace evidence examinations when other forensic capabilities are inconclusive. On 04 July 2003, Manuel Gehring kidnaped his daughter and son in New Hampshire. Fearing a possible custody fight with his ex-wife, Gehring shot his children and drove across the country. Six days later, Gehring was arrested in California. He confessed to killing his children and told investigators he buried the children's bodies in a remote location off Interstate 80. Gehring provided detailed descriptions about the gravesite but maintained that he could not remember where he buried the bodies. Investigators focused search efforts on a 700-mile stretch of I-80 west of Grove City, Pennsylvania, where Gehring had purchased digging implements from a Home Depot, to Iowa City, Iowa—a diverse geographic area containing dramatic vegetative changes. Later stages of the investigation intensified search efforts in western Ohio and eastern Indiana. Gehring killed himself in his prison cell in February 2004 before he could stand trial and before the bodies of his children were located.

Geologists in the FBI's Mineralogy group received the two shovels and pick axe Gehring purchased in Grove City, Pennsylvania as well as debris recovered from the undercarriage of Gehring's van. They were tasked with determining the source of the soil recovered from the implements and from the van. Mineralogical examinations on the limited amount of debris recovered were inconclusive. The debris contained common minerals (quartz, calcite, etc.) and road debris, indicative of an urban/highway environment (reflective paint beads, paint chips, asphalt, etc.).

The FBI proceeded with a pollen examination with the assistance of the USGS in Reston, Virginia. Pollen was extracted from the soil recovered from the tools and from debris recovered from the undercarriage of the van. USGS palynologists processed, identified, and analyzed the pollen assemblages from each item. Dominant and diagnostic pollen components were identified with the assemblages dominated by tree pollen. Several wetland plant species were also represented. These assemblages were referenced against modern pollen distribution maps. The analysis concluded that the most likely source for the pollen assemblages recovered from the items was a region of northeastern Ohio, between Cleveland and the Pennsylvania border—a geographic area opposite from where previous search efforts were focused in the state.

In early December, 2005, a search volunteer from Akron focused her efforts in an area along I-80, Route 8, and Route 303 in northeastern Ohio. Stephanie Dietrich and her dog Ricco based their search on information from investigative reports, including FBI interviews and documents. On December 1, they located the Gehring children's bodies in graves located in a remote area near Hudson, Ohio. The gravesite matched the physical description Gehring gave investigators. Geographically, Hudson is located in northeastern Ohio, approximately 30 miles southeast of Cleveland and 55 miles west of the Ohio-Pennsylvania border.

Conclusions

As demonstrated with our case example, forensic palynology can be a powerful investigative tool. Nearly two centuries of research on pollen morphology (see Traverse 1988) has led to description and illustration of pollen of most plant species; combined with knowledge of the distribution and ecology of the source plants, pollen analysis provides a valuable tool to identify source plant communities, source environment, and likely source areas for evidentiary material.

However, without increased efforts by the forensic science, law enforcement, and the legal communities, the discipline of forensic palynology will remain an afterthought. Fostering relationships between forensic scientists and palynologists can eliminate some of the factors that have limited the use of forensic palynology in the United States. Education, awareness, and accessibility remain the keys to unlocking forensic palynology's full potential in casework.

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Appendix A: A Pollen Primer

Pollen and spores are ubiquitous in the environment because of the prolific production of pollen by plants. The wide variety of shapes, sizes, and surface characteristics of pollen grains that can be used for identification and the easy transfer of pollen between people, places, and objects makes them ideal for forensic trace analysis. The walls of pollen grains are composed of compounds that are highly resistant to most forms of decay enabling recovery from a variety of evidentiary items and reference samples.

Production and dispersal

Pollen production varies depending on the plant's method of pollination and dispersal, which include self-pollination and pollination by water, animals, and wind (Faegri et al. 1989). Pollen production varies widely among pollination methods. Self-pollinated plants typically produce <100 grains per anther (the organ at the upper end of the plant's stamen that secretes and discharges pollen); animal/insect-pollinated plants typically produce less than 1,000 grains per anther and plants with wind-dispersed pollen can produce between 10,000 and 100,000 grains per anther (Milne et al. 2005).

Pollen dispersal methods also play a large role in determining distance of pollen dispersal. Wind-borne pollen typically is transported between 25 meters and 2 kilometers from the source (Milne et al. 2005) with a maximum dispersal limit of 50 to 100 kilometers from the source (Faegri et al. 1989). Animal- and insect-borne pollen typically disperses near the source plant. The presence of this type of pollen in a forensic assemblage provides more site-specific evidence than pollen from wind-dispersed species because this method relies on contact for dispersal (Eyring 1996).

Pollen Morphology

In the most general sense, pollen grains are ellipsoids in shape with rotational symmetry enabling the identification of the polar axis and the equatorial plane of the grain (Kapp et al. 2000). Grain sizes vary from between 5 µm to greater than 200 µm, with most grains ranging between 20 µm and 50 µm (Kapp et al. 2000). Great variation exists in the morphological features of grains of different taxa (Fig. 1). In addition to the size and shape of the grain, palynologists compare the shape, number, and arrangement of wall apertures (openings), as well as the surface structures and sculpturing that can vary from smooth surfaces to rod-, club-, or spine-shaped ornamentation.(Faegri et al. 1989; Kapp et al. 2000).