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Kevan A.J. Walsh,[1] *M.Sc. and Mark Horrocks,*[2,3] *Ph.D.*

Palynology: Its Position in the Field of Forensic Science

ABSTRACT: Here we examine the current state of palynology in the field of forensic science. Forensic palynology is discussed with reference to other forensic disciplines to help understand what is required for its progress. Emerging developments are also discussed. Palynomorphs potentially deliver excellent trace evidence, fulfilling the requirements relating to the transfer, persistence, and detection of such evidence. Palynological evidence can provide very powerful investigative and associative evidence. Despite this, the application of palynology to forensic science has had mixed success. There are many anecdotal stories where pollen evidence has had spectacular successes. But it is extremely underutilized in most countries because it is labor-intensive and requires considerable expertise and experience, there is a lack of control over sample collection and inadequate resourcing and funding, and its crime-solving power is not well known. Palynology has been applied to forensic problems in an unstructured way, resulting in a lack of formalized discussion of the underlying principles. As there is renewed questioning of the acceptability of most evidence types in the current legal environment, there is a need for the establishment of palynological evidence through validation-type studies and experimentation, and the implementation of independent proficiency testing.

KEYWORDS: forensic science, forensic palynology, pollen and spores, trace evidence

Palynology involves the study of pollen grains and spores, collectively known as palynomorphs, and commonly all included in the terms "pollen" and "pollen types." Although the science has been around for a long time and there has been no significant change in the basic analysis and examination of palynomorphs, the application of palynology to crime investigation is relatively recent.

In a 1930 paper on the forensic analysis of dust traces, Locard (1) listed pollen as one of the types of botanical dust debris. It was a very cursory mention however, noting that "the wind likewise transports pollen dust, whose constant presence is noted on garments and on the body during the summer season." No casework examples were given; however, he was more effusive on the subject of mushroom spores, of which he had made some study. He noted that "the spores collected in dust are often quite characteristic and permit us up to a certain degree to determine what the bearer has recently been doing, such as walking in a wood, collecting mushrooms, etc."

It is surprising perhaps that it took until 1959 before the application of palynology to crime was reported (2–4). One of these cases was in Europe in 1959. The presence of pollen in soil, taken from the shoes of a suspect, was used to show an association between the suspect and an area of forest where a body had been dumped. A man had disappeared on a journey down the Danube River. Although investigators did have a suspect with a motive, they had no body or crime location. A palynologist, Wilhelm Klaus, examined mud from the suspect's shoes. This showed the presence of modern pine and alder pollen together with 40-million-year-old fossil pollen. By reference to geological and vegetational maps of the area, a likely location along the Danube Valley was identified as the source of the mud and therefore as the area where the suspect had been. Upon being presented with this information, the suspect confessed to the crime and indicated the whereabouts of the body.

Since the application of palynology to forensic science in 1959, there have been numerous reports of other applications. These have helped highlight the versatile nature of the evidence and to establish the field of forensic palynology as one that is generally accepted in the scientific community. However, the reports have mainly been anecdotal accounts rather than scientific studies to establish the field in its own right as a scientific discipline. It has also been noted that forensic palynology has not been universally adopted in crime laboratories like most other evidence types (4).

This paper examines the current state of forensic palynology, emphasizing where the discipline fits within the general field of forensic science rather than an overview of the discipline itself. The state of forensic palynology is discussed with reference to other forensic disciplines to help understand what is required for its progress. Emerging developments are also discussed.

Forensic Palynological Methods

Many types of forensic samples such as soil, clothing, and illicit drugs typically contain palynomorphs and these samples are chemically treated to concentrate the palynomorphs. The pollen from each sample is then mounted on a glass slide for examination with light microscopy (400–1000×), and the various pollen types are identified and counted. The taxonomy of pollen and spores is very well known. Typically, a total of at least 100–200 palynomorphs are manually counted per sample, and percentage tables or graphical diagrams produced. If samples are being compared with one another, usually the types and amounts of pollen from each sample are compared between samples. At this stage, statistical analyses are rare; palynologists doing forensic work mostly assess the data based on knowledge and experience. For specific experimental

[1]Institute of Environmental Science & Research Ltd., Private Bag 92-021, Auckland, New Zealand.
[2]Microfossil Research Ltd., 31 Mont Le Grand Rd, Mt Eden, Auckland, New Zealand.
[3]School of Geography, Geology & Environmental Science, University of Auckland, Private Bag 92-019, Auckland, New Zealand.

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details and general overviews of forensic palynology, readers are referred to other publications (3,5–8).

Palynomorphs as Trace Evidence

Palynomorphs have the potential to deliver excellent trace evidence. They are small, transferred readily between objects, can persist on objects for considerable periods, and can be recovered and identified. Therefore, they fulfill the requirements relating to the transfer, persistence, and detection of trace evidence (9,10). If there are reservations, they relate to interpretation in general and more specifically, the lack of experimental data on matters such as transfer and persistence.

Detection and Persistence

Pollen and spores are microscopic, being *c.* 5–200 μm in size, but more typically *c.* 20–70 μm. Although they may at times be visible as an accumulated mass, they do not present to perpetrators an obvious connection to a crime scene as would, say, blood. Perpetrators would not typically know that pollen is on their clothing, especially that which has been transferred indirectly through the presence of soil.

Palynomorphs are very persistent, both in retaining their form and in being retained, say, on clothing. Although the protoplasm within the cell wall may decompose quickly, the wall is extremely resistant to degradation and may be well preserved depending on the environment. Optimal preservation of organic material occurs in permanently wet or dry environments. Pollen grains may thus be preserved for millions of years. Even in less favorable environments (i.e., that undergo alternate wetting and drying), such as well-drained soils, pollen may persist for many years. This means that a soil may develop a diversity of pollen types, reflecting the vegetative history of that location. Even a soil supporting little or no vegetation may have potential palynological diversity as a result of what has grown in the locality in previous years, or by what has drifted to the area from elsewhere.

In a forensic context, the persistence of pollen as transferred evidence is important. Being tiny, palynomorphs have the potential to be caught in clothing fibers or other material. They can therefore persist on the material for a considerable time, facilitating later recovery. When palynomorphs are present as a component of other material (e.g., soil), their persistence is related to that of their "carrier." Typically, carriers such as soil do have considerable persistence by adhering to the fibers of clothing or being lodged within the sole patterns of shoes.

Identification and Abundance

Palynomorphs have often complex and diverse cell wall structures, making them very identifiable to a particular family or genus, if not species. This variability is manifested in their size, shape, apertures if present and wall pattern. As a result, any sample may contain a large number of very diverse and potentially identifiable pollen types. As more pollen types are identified in a sample, the more distinguishable the sample becomes from other samples that each reflects their different current and historical vegetation.

Palynomorphs are typically abundant, as is required of them in their reproductive role. This abundance means that they have much potential as trace evidence. Pollen and spores have different sexual functions. The pollen grain is the male gamete, being dispersed from the anther (male part) of seed plants to reach the pistil (female part), where fertilization occurs. On the other hand, nonseed plants such as ferns produce spores, which are asexual reproductive bodies that form new plants without fertilization. To ensure fertilization or regeneration, pollen and spores are generally released in large quantities, depending on the dispersal mechanism and proximity between the male and female parts or between plants of the same species. Therefore, the presence of palynomorphs in great quantities on or in some object such as soil means that they can be transferred to another object, such as the shoes or clothing of a suspect, in large numbers, increasing their chance of detection by the forensic scientist.

Dispersion

The production and dispersal of pollen is well covered elsewhere (e.g., 3,5,11,12), but it is useful to discuss the differences as they underline why some plant types may be more prevalent as "pollen evidence" than others. There are four general types of pollination, categorized by their method of dispersal of pollen, as follows:

1. water pollination where pollen is dispersed between submerged plants by water currents;
2. self pollination where the anthers and stigma mature at the same time, requiring movement of pollen over only a short distance;
3. animal (mostly insect) pollination, where the anthers and stigma mature at different times, requiring transportation of pollen by unwitting "carriers"; and
4. wind pollination, which is a very inefficient dispersal method requiring the production of large quantities of pollen to ensure arrival of some pollen grains at their destination.

The other significant feature of pollen dispersal is the concept of "pollen rain." For animal-pollinated plants, pollen grains are mostly produced and deposited in relatively low numbers in the area immediately beneath the plant. Whereas for wind-pollinated plants, which have much higher pollen production, the pollen is deposited at varying distances from the plant, depending on the height at which the pollen is released, the wind speed and direction, and the size, mass, and aerodynamic shape of the pollen grains. It is reported that 95% of wind-dispersed pollen falls within 2 km of the parent plant (13). Therefore, the presence of wind-dispersed pollen in a soil sample does not necessarily indicate the immediate presence of a particular species of plant from where the sample has originated. However, the presence of animal-dispersed pollen would indicate the immediate presence of a particular species of plant. More than half of the world's flowering plants use animal pollination.

One example where this differentiation between animal and wind pollination was important occurred with the investigation of a plane crash (14,15). The crash remains had been stored at an airport storage site. A later investigation of the cause of the crash queried whether a minute pellet within part of the fuel component of the plane could have been a factor in the crash. The pellet was analyzed and found to consist predominantly of pollen from insect-pollinated plants, all of which were found in the vicinity of the storage area. Pollen grains from insect-pollinated plants would not be expected to be airborne in any significant numbers where they might be filtered into the fuel system while the plane was flying. The inference drawn from these findings was that the insect-borne pollen had accumulated postcrash, in the storage area.

Investigative Evidence

Palynological evidence can provide very powerful "investigative evidence," as is evidenced by the many anecdotal reports of information gathered from an examination of pollen assemblages within samples. A pollen assemblage is the total number of pollen and spores identified in a sample. A classic example of the use of palynology as investigative evidence is the case outlined earlier, whereby a man had disappeared on a journey down the Danube River in 1959. The location of the body was determined from analysis of pollen in soil from the suspect's shoes. This determination relies on knowledge or survey information of particular regions. In this case, it was geological and vegetational maps.

Pollen examination of a sample can also yield information relating very specifically to a particular crime scene or whatever localized environment the sample originated from. Despite a pollen grain being microscopic, the information that is provided by an identification of the species from which it is derived can be projected onto a much more tangible level. This information may be the identification of a visibly large or conspicuous type of plant species that can be located at a crime scene. Therefore, the presence of large quantities of pollen from a particular plant on clothing strongly points to contact with or close proximity to that particular plant. This information can be used to assist in identifying a likely crime scene location from the location of the particular plant identified, or it can allow reconstruction of a crime or movement of certain people, by understanding the interaction with certain plants.

Associative Evidence

As well as investigative evidence, palynological evidence can also provide very powerful "associative evidence." Palynomorphs are ubiquitous in the environment and therefore nearly every sample will have some background level of pollen and spores. However, the potential diversity of pollen types within a sample means that powerful evidence can be provided when similar assemblages of palynomorphs are found in comparison samples.

One example where palynological evidence provided very powerful associative evidence was the correspondence observed for the pollen assemblages of clothing items taken from a suspect who lived in a coastal area of New Zealand and the clothing from a victim who had been shot in a mountainous area (16). The vegetation around the body consisted of higher altitude plants, particularly silver beech trees. Pollen from the victim's clothing reflected the higher altitude environment that it had lain in for many weeks. Clothing from the suspect contained a similar assemblage of pollen. This provided crucial evidence as the defendant changed his story in court, to admit that he had been in the general area where the body was found, despite earlier claims to the contrary. In an investigative manner, the identification of silver beech pollen on the victim's clothing allowed the police to focus on a tangible means of establishing if the suspect had an innocent or alternative explanation for the presence of such pollen on his clothing. The presence of silver beech in particular areas is very easily determined. It was determined that there were no silver beech trees in the city the suspect lived in, except for some botanical gardens, which the suspect had not visited or been near. Nor were there silver beech trees in other areas away from the city, where it was known the suspect had visited. Therefore, the palynological evidence in this case proved an important potential association between the suspect and crime.

In another example, the association of a suspect with a sexual assault scene was determined from the presence of a large percentage of pollen from one plant genus, *Hypericum* (17). The offender was seen to brush against a *Hypericum* plant when leaving the premises. The suspect's jacket, pants, and shirt had 24%, 14%, and 27.5% *Hypericum* pollen, respectively. The pollen grains still had their cell contents preserved and many were clumped, indicating that they were fresh. This presence of large proportions of fresh *Hypericum* pollen on his clothing could only have occurred from recent direct contact with a *Hypericum* plant.

Absence of Evidence

Depending on the type of pollination of the plants within a particular environment, one would expect that there would be a similar pollen assemblage for samples taken from a particular area within a crime scene. Therefore, the absence of particular assemblages may well prove convincing evidence of a lack of association with a crime scene. The often well-publicized "success stories" of forensic science typically relate how the science has been used to lead investigators to the offender or to conclusively associate the accused with the crime. However, a lesser-publicized but extremely important role of forensic science is to correctly dissociate an innocent suspect from a crime. Palynological evidence has a vital role in this. Although as an expert witness the palynologist may struggle with assigning evidential significance to an observed correspondence of palynological assemblages, there is rarely ambiguity associated with contrasting assemblages. For example, consider a situation where the police believe that there is an association between a suspect and a cannabis plantation. A comparison of the pollen assemblage of a cannabis sample belonging to the suspect with the environment of the cannabis plantation could assist the determination of whether or not there is any association between the suspect and plantation. If the subsequent analysis of the (non-cannabis) pollen in the suspect's cannabis sample shows that there is no commonality with the plant environment of the plantation, then this palynological evidence would clearly support exoneration of the suspect. At times there may be questions about the relevance of certain samples, but often the difference observed between palynological samples from different locations is so stark that the only conclusion that can be drawn is exclusionary.

As well as using palynology to establish the innocence or at least the nonassociation of a person with a particular crime scene, it may also assist in disproving a particular alibi. In an example of the latter, which also demonstrates the potentially precise localization of particular assemblages, an alleged rape victim described events taking place in an alleyway consisting of an area with a mostly bare soil surface and small overhanging *Coprosma* trees between two buildings (18). The alleged rapist stated that no sex occurred and that soil on his clothing was from the ground next to a driveway *c*. 7 m from the alleyway. Although there was some similarity in pollen assemblages for the alleyway and driveway samples, as expected for samples taken from such a localized area, there were significant differences in percentages and types of pollen. The pollen assemblage of the soil sample from the suspect's clothing showed a marked similarity with the soil from the alleyway, being particularly high in *Coprosma* pollen and low in grass pollen. In contrast to the driveway, the alleyway had a dense canopy of *Coprosma* bushes and little grass. This evidence strongly supported the victim's assertion that all activity had taken place in the alleyway, supporting her claim of sexual assault in this particular spot.

Interpretation of Evidence

Forensic palynology is less well developed in the interpretation of the information arising from the comparison of samples. This probably reflects its relative immaturity as a field of forensic science, the manner in which the forensic aspect has been applied on an "as-required basis," the lack of application of palynology in many forensic laboratories, and the sheer difficulty of the task. Some information may be available locally regarding the distribution of pollen types in soils from awareness by the particular scientist, through actual sampling or simply through knowledge of the local vegetation. Alternatively, such knowledge may be acquired through studies unrelated to forensic applications, such as ecological studies.

Even less well researched is the modeling of transfer and persistence of palynomorphs in different forensic situations. Part of the reason for this may be that there is such a diverse manner in which palynological evidence may be transferred that all possibilities cannot be reasonably addressed. For example, the analysis of ropes left in an outdoor environment will have different issues regarding pollen assemblage variation with locality and persistence than would situations involving the transfer of soil onto shoe soles. An attempt has been made to initiate research in this area, with studies regarding the expectation of pollen assemblage transfer to shoe soles depending on soil location and depth (19,20).

The lack of full-time palynologists within forensic laboratories is a contributing factor to the lack of development of methods of interpretation. Palynologists who are called on to do forensic work are often employed as researchers or investigators of other aspects of palynology. Therefore, they often do not have the full-time commitment and resources to apply to forensic tasks to enable the development of their own knowledge database or actual survey work.

Forensic palynology may utilize similar methods of sample preparation and examination as other palynological applications. In any pollen assemblage examination, the actual identification of pollen grains that are present is often a major task of the examination. Where forensic palynology is different to most other palynological investigations, is that there is less emphasis on the identification of pollen types and more emphasis on the comparison of pollen assemblages between samples. Although identification of some of the pollen types may remain unknown, their common presence still points to association. It is the comparison step between pollen assemblages that is often the most important when it comes to delivering results in the courtroom setting, yet it is an area that is not really well understood and not well studied.

The comparison of pollen assemblages is fraught with many difficulties. Each sample is just that, a sample. Simple variation due to sampling means that perfectly corresponding assemblages can never be obtained. The percentages of pollen types will never be exactly the same. A particular type of pollen that is in very low abundance in a particular area may not be represented in a sample taken from that area. Different samples will inevitably be taken from slightly closer to, or further from, a particular plant meaning that the pollen rain will differ for those two samples, resulting in different abundances. Therefore, studies of sample variation from localized areas become as important as studies of sample variation from very different locations.

To an extent, the modeling of general pollen distribution in the environment is well known. The differences between pollen distributions from plants whose pollination is, say, wind-assisted versus animal-assisted are well understood. However, from a forensic point of view, the effects of this on actual percentages of pollen types within an assemblage are not well known. The task becomes extremely complex when there are factors to be considered such as the diversity of plants, the variation in their quantities of pollen production, the timing of pollen release, the differences in their modes and rates of pollen dispersal, and the differences in climate and topography for each local environment.

Other important forensic matters, such as the probability of transfer of pollen upon contact or the probability of transfer resulting from being in the near vicinity of a pollen-bearing plant, are neither known nor studied. As well as transfer, aspects of interpretation relating to the persistence of pollen on clothing are equally important. But again, the wide range of substrate types that pollen can be recovered from, make this task very difficult to tackle.

In many aspects, forensic palynology has strengths that make it a great candidate for forensic trace evidence. What limitations exist may be overcome or lessened by application of more theoretical studies. To assist in determining the strengths, weaknesses, and future direction of forensic palynology, it is worthwhile comparing it with other forensic techniques.

What Can Forensic Palynology Learn from Other Forensic Disciplines?

The Frye Test

For many years, courts in the U.S. reviewed the admissibility of scientific expert evidence according to the Frye test (21). This set a rule that scientific evidence was allowed if it "gained general acceptance in the particular field in which it belongs." This "general acceptance" was quite a restrictive standard, particularly so for new scientific work. Although new work might have been well studied and properly validated, if it had not gained general acceptance, expert evidence relating to it would not be allowed.

It could be argued that forensic palynology has met the Frye standard. There are many published instances of the application of palynology to crime (e.g., 3,6,8). The presentation of such applications at mainstream palynology conferences and their publication in mainstream science journals would support this. However, there is a paucity of information on the application of forensic palynology in major texts on the general subject of pollen analysis.

It might even be argued that one of the first presentations of palynological evidence did not result in agreement (3). The disagreement was between expert palynologist witnesses called for the defense and prosecution, who agreed on the pollen compositions of the samples, but differed in the interpretation. It was a significant indication for the need for future study, that the technical component of the science was not in question, but rather the interpretative aspects were debated. The case involved the murder of a woman in 1959, and the issue became whether or not she was killed where her body was found. One witness argued that the difference in assemblages between the victim's clothing and the surrounding soil pointed to her having been murdered at a different location. The opposing witness argued that the difference was a result of her having been killed a month before the body was found, with the different pollen assemblages between the body and the surroundings due to seasonal variation. A difference of opinion such as this should not be seen to undermine the entire validity of the field, but it did mean that the evidence did not get off to the best start.

The Daubert Criteria

In 1993 the Frye test was changed to a more demanding standard (22). The relevant ruling set the trial judges as "gatekeepers" of expert evidence and the court set four criteria by which scientific testimony must be evaluated before it can be admitted:

1. General acceptance in a particular scientific community.
2. Peer review and publication.
3. Known or potential error rate.
4. Testability of scientific principle.

The first criterion is effectively the Frye test, so the addition of three others has enhanced the standard. The first and second criteria obviously go hand-in-hand. The third criterion, error rate, is one that makes particular sense from the point of view of the trier of fact, the court. If an expert witness forms an opinion significant in determining guilt or innocence, a simple way for the court to work out how much weight to attach to it is to know how often the witness is right, when giving such evidence. A simple enough question but one that belies the difficulty associated with assessing this factor.

The error rate could be assessed at many levels. There might be errors in the handling or preparation of the samples, in the identification of pollen grains to a genus or species, or in the final conclusion regarding similarity between samples. To determine an error rate, the question really needs to be a "yes" or "no" question. The relevance becomes somewhat muted when the answer is of the "could have" kind. Therefore, the error rate aspect works best with the classic identification sciences. With fingerprints, a question might be "was that print made by the defendant?" The answer is typically "yes" or "no."

By some series of tests, an error rate could conceivably be determined. Most scientists in the identification sciences might contend that their error rate is zero. However, this seems not to be the case. One way in which error rate can be determined is via proficiency testing, which is standard for most mainstream forensic disciplines. What this testing has shown is that error rates are not zero and in some cases are surprisingly high (23,24). The reasons for these errors may be disputed. For example, some proficiency testing is given to trainee scientists and any erroneous reporting by them may reflect their level of training. Others may be the result of language difficulties from non-English speaking participants, and some may be the recording of an inconclusive result as an error when the answer is known to be either "yes" or "no." However, at least some errors are the result of transcription mistakes, mixed samples, or outright erroneous conclusions. It also may be contended that proficiency testing is a measure of an individual working in the absence of the normal laboratory procedure of quality assurance and peer review. Any errors that might slip through, such as transcription errors, might well have been picked up during an administrative or technical review. Despite protestations regarding the use of proficiency testing as a measure of an error rate, in most cases it is the only independent measure available.

The implementation of independent proficiency testing into the field of forensic palynology is required. The scoring of such proficiency tests is fraught with difficulty however, particularly regarding interpretation. Consider the provision of a proficiency test where, say, two replicate soil samples are taken from one location and another soil sample is taken from a different location. If we consider one of the replicates as a "control" sample and the other two samples as "recovered" samples, then the correct answer would be that the replicate recovered sample did come from the same source as the control sample and that the other, different recovered sample did not come from the same source as the control sample. However, such a closed set of alternatives is atypical. Depending on the degree of correspondence of the respective assemblages regarding the variety and rarity of pollen types present, one might draw the conclusion that "the pollen evidence very strongly supports the proposition that the (replicate) recovered sample came from the same location as the control sample." Clearly this may be the correct conclusion. Indeed, if there was mostly grass pollen (a generally very common, widely dispersed pollen type) present in the control and replicate recovered sample, then any conclusion of certainty regarding the origin of the samples would be improper, despite being strictly correct.

There also needs to be awareness of errors of pollen type identification, although the effect of this may be lessened by the comparative nature of the analysis. There may need to be conducted some assessment of pollen type identification error. Generally the identification of pollen types is difficult, depending heavily on the scientist's experience and access to an extensive pollen sample and literature reference collection. Species of a particular genus often cannot be distinguished and this is well recognized. However, it is interesting to note the potential dispute over the correct identification of some pollen types, which may reflect an overly confident level of precision. An example of this relates to the identification of pollen recovered from the Shroud of Turin. In that case, many of the species listed as indicating a Palestinian geographic source (supporting authenticity) are generally regarded as being capable of identification only to genus level (12).

The fourth criterion of the Daubert standard, testability of scientific principle, relates to the scientific foundation of palynological evidence, requiring a transparent method of scientific examination, analysis, comparison, evaluation, and interpretation. It is fair to criticize the field of forensic palynology for not having ever propounded the scientific method of this field. It is indirectly addressed in many publications through discussions about what the question is to be answered. Interestingly, the principles and basic assumptions have been discussed for the general field of palynology (11,12), but it is only recently that questions have been asked as to its robustness (25).

It is useful to illustrate what is required of forensic palynology to meet the Daubert standard of testability of scientific principle by comparing to other forensic fields. Why reinvent the wheel, when the legal and scientific issues have been traversed by analogous fields? However, fields that have undergone some degree of critical self-analysis are typically those from the "identification sciences." These include fields such as fingerprints and firearms identification, where identification refers to the source of the mark, whether it is a fingerprint mark or the marks imparted to a bullet as it passes through the barrel of a firearm. Questions relating to source in these fields tend to yield classic "yes" or "no" answers. Conversely, comparisons in forensic palynology may not be so clear-cut. Although a significant difference between pollen assemblages would result in an outright exclusion, can a particular assemblage be ascribed to a particular origin or source material to the exclusion of all others? The answer is most probably "no" (e.g., 26). The lack of a definitive answer to this problem however, highlights some of the weaknesses in forensic palynology. There is not objective or sufficient data available to make such decisions and therefore the decision-making process is fundamentally subjective.

Subjective decisions are not synonymous with bad decisions. It has been construed by the courts, however, that subjective decisions tend to have more "art" attached to them than "science" (27). The admissibility of some opinion evidence has been challenged and not accepted on this basis (28,29). This has happened when the examiners have been unable to demonstrate the underlying science (27). This has led to justifications of forensic identifications by examiners who state that "I know a match when I see one," for example with regard to "pattern matching." Although typically applied to patterns such as fingerprints or marks on bullets or other marks made by tools, the term "pattern matching" could also apply to patterns of pollen assemblages, where the pattern comprises various amounts of different pollen types. The various challenges to other forensic disciplines have allowed recognition of what is required to meet the challenges. These include the following:

1. Formalize the science underlying the forensic comparison.
2. If a subjective decision is made, articulate the science that backs up the subjective decision-making process.

3. Apply objective criteria if they are available, or design experiments to allow determination of objective criteria.

What is the Question?

The determination of what question needs to be answered, in relation to a forensic palynological examination or comparison, has rarely been discussed in the literature. An exception is for the forensic examination of dust samples (30). Dusts of outdoor origin were defined as consisting primarily of mineral grains, pollen, spores, plant cells and tissue, leaf hairs, soot, and charred particles. Three main categories were proposed for questions that can be addressed by the analysis of dust:

1. Is there evidence of likely contact, in some combination, among a victim, suspect, and the scene of crime?
2. Can the origin of a dust sample be discovered, or its location described, from its composition?
3. Is it possible to determine a person's occupation from an examination of the dust on clothing?

Clearly the first question relates to associative evidence, whereas the other two relate to investigative evidence. The following is offered as a primer for establishing the principles of forensic palynology. There may be different applications for different forensic situations, but keeping it simple, consider the example whereby pollen evidence is transferred from the crime scene to the clothing or shoes of the perpetrator of the crime. The ultimate question to be answered is "could the pollen recovered from the clothing and shoes be from the crime scene?" This is a question of common source. Therefore, the question could be more generally put in terms of "do these two samples (recovered and questioned or control) share a common origin or source?" Principles underlying the response to this question would include the following:

1. A sample has a pollen assemblage that reflects its current and historical vegetational environment.
2. Samples taken from different sources will have different pollen assemblages reflecting their different current and historical vegetational environments.
3. Samples taken from the same source will share similar pollen assemblages.
4. There may be small differences between pollen assemblages taken from the same source.

With the type of framework outlined above, the comparison can be conducted with knowledge about what needs to be tested. Assumptions that may not have been thoroughly tested can now be identified to be tested with proper experimental design, and the significance of a result can be assessed. Most workers probably follow this type of approach, but they might not specifically formalize their thinking.

Drawing a Conclusion

If a comparison shows a difference of pollen assemblages beyond that which the scientist has previously encountered, then the finding would be that the two samples do not share a common origin. Inherent in this finding is that the examiner knows what differences to expect between replicate samples from the same source. If the scientist finds that there is a similarity between samples, then the finding would be that the two samples could share a common origin. The significance of this finding might be determined with cognisance of how common the particular pollen assemblage is and what minor differences between the samples were observed. This assessment would be made from the scientist's previous experience.

We have previously discussed articulating the conclusion (31). We supported the use of a Bayesian approach to the formulation of a framework in which to assess the evidence. This proposes that two alternative hypotheses be tested and the weight or significance of the evidence is assessed by consideration of the likelihood ratio, which is the ratio of the probabilities associated with each competing hypothesis. The numerator and denominator of the likelihood ratio are therefore probabilities conditional on complementary scenarios.

If one considers the evidence to be the pollen assemblage observed in the sample recovered from the clothing or shoes of the perpetrator of the crime, then the numerator is the probability of obtaining that evidence (observed pollen assemblage) if the sample does indeed come from the crime scene. One way of looking at this is to consider that if we took many replicate samples from the crime scene, we could assess the variation of pollen assemblages that exist with such sampling. With close agreement between the recovered sample and the replicate samples from the crime scene, a high probability would be assigned to the correspondence observed. Therefore, the numerator effectively assesses the quality of the correspondence observed between the recovered sample and the replicate samples from the scene.

The denominator is the probability of obtaining the same evidence (i.e., the pollen assemblage observed in the sample recovered from the clothing or shoes of the perpetrator of the crime), if the sample does not come from the crime scene. Therefore, this is an assessment of how likely it would be to obtain such a pollen assemblage from another location unrelated to the crime scene, i.e., how common is such a pollen assemblage?

When the numerator and denominator are compared as a ratio, a large number for the likelihood ratio denotes strong evidence of association between the sample recovered from the clothing and the crime scene. This occurs if the numerator is high (resulting from a high quality of correspondence between the recovered sample pollen assemblage and the crime scene pollen assemblage) and if the denominator is low (because the observed pollen assemblage is uncommon). Without survey data assessing how common pollen assemblages are, and without replicate studies of pollen assemblages from known sources, this calculation of the likelihood ratio cannot be enumerated. However, it has been suggested that the likelihood ratio can be subjectively assessed (31).

One of the challenges for the future of forensic palynology is to develop a means of objective assessment. A start has been made on this. Articles which have approached the question of how common is a particular pollen assemblage (i.e., denominator of the likelihood ratio), have been published (e.g., 19,32,33). However, there are fewer articles discussing the expected correspondence between pollen assemblages known to be from the same source (i.e., numerator of the likelihood ratio) (e.g., 20,32,33).

A complicating factor is the possibility of contamination of samples through mixing pollen assemblages from different sources. For example, soil in the soles of shoes may be a combination of soil from walking in mud at a crime scene and from walking in mud from a location unrelated to the crime scene. Other complicating considerations, possibly specific to forensic palynology, include the differences expected with seasonal variation. Other work that needs to be addressed, for a complete interpretation of palynological evidence to be made, includes studies relating to the transfer of evidence (from crime scene to shoes or clothing) and the persistence of evidence (how long the evidence is expected to remain on the clothing of the offender).

What Does the Future Hold for Forensic Palynology?

Promotion of the Discipline

Forensic palynology has had some spectacular successes with the application of the science to forensic problems (e.g., 4,8,34). However, its application as a routine analysis in forensic laboratories is very uncommon. In many instances, the palynologists consulted regarding forensic work are employed as palynologists for nonforensic applications. There may be many reasons for this situation (4). Palynology is very labor-intensive and requires considerable expertise and experience. It may be difficult to find experienced palynologists prepared to commit to forensic work, with the extra demands of court appearances, which are foreign to most scientists. A lack of control over sample collection and inadequate resourcing and funding are other factors that make it difficult to employ full-time forensic palynologists.

It is also recognized that ignorance of the power of palynology to solve crime may be another factor in its general lack of application. This was identified in a survey of law enforcement agencies in the U.S. (4,34).

Use of the Scanning Electron Microscope

Traditional palynology has relied very heavily on microscopy as the primary means by which palynomorphs are identified. The application of the scanning electron microscope (SEM) to the discipline has allowed for imaging of pollen grains with far greater definition than was previously possible using light microscopy. The SEM cannot be hailed as a recent technological advance, having been routinely available from the 1970s, but its application on a more routine basis might be considered an advance. However, the use of a SEM typically would increase the labor required for a technique that is already highly labor-intensive. Therefore, despite its imaging advantages, it is unlikely that the SEM would be routinely utilized for forensic analysis.

Automated Imaging

The greater definition of images obtainable with a SEM has led to research into applying an automated method of pollen analysis (35,36). This has been reported as being very successful at identifying pollen grains, although if the reasons described above regarding the nonutilization of the SEM remain valid, then the extended use of the SEM for automated image analysis would also appear an unlikely candidate for forensic applications. For automated image analysis to be implemented, it would require a thorough, fast, and reliable application to offset the barrier to use of the SEM in the first place.

It can be envisaged that automated image analysis would be best applied to quickly generating relative abundances of pollen types within a pollen assemblage of a small number of well-documented pollen types. However, with forensic samples it is important to recognize, count, and document the presence of rarely occurring pollen types within the overall pollen assemblage that may be dominated by large amounts of possibly common and readily identifiable pollen types (such as those of grasses). It is through awareness of the rarer types within the assemblage that discriminating power is developed. Therefore, any image analysis needs to ensure that the less frequent pollen types are registered.

Objective Discriminant Analysis

Another area of forensic palynology that is waiting for development is some form of objective discriminant analysis (33). Presently the palynologist attaches weight to the comparability between assemblages depending on the relative amounts of different pollen types, but also places emphasis on unusual or atypical pollen types present within the assemblages. An assessment of the latter includes information or knowledge regarding the relative frequency of the parent plant and its mode of pollen dispersal. In this way animal-pollinated plants take on particular significance, for example, because their pollen dispersal onto the ground is expected to be localized to the immediate vicinity of the plant. Conversely, wind-pollinated plants are typically over-represented in pollen assemblages. Therefore, any form of objective analysis has to assimilate that information to formulate a weighted or step-wise approach to develop a reliable means of discrimination. The challenge is to develop a method that can compare with the assessment made by an experienced palynologist who simply runs a trained eye over graphic percentage diagrams of pollen assemblages.

DNA Analysis

A most promising application of new technology relates to the comparison of pollen assemblages by their DNA profile. The application of DNA analysis to pollen has been reported (e.g., 37,38), but there have been no forensic applications reported thus far, although research into this area has been conducted (39,40).

DNA profiling has established itself as a powerful and accepted technique as applied to the comparison of bodily fluids. There has been considerable effort expended to develop databases or surveys of DNA profiles of many human populations and there has developed very sophisticated tools for interpreting the data. The application of DNA profiling to forensic palynology can benefit from the lessons learnt already.

The potential exists to apply DNA profiling to individual pollen grains (38) and demonstrate some relationship between the DNA of a particular grain or grains to an individual plant at a crime scene, depending on the variability of DNA between plants within a species and the development of appropriate primers for DNA amplification. The tRFLP technique has been applied to the DNA analysis of pollen grains (40). In the near future this might have some application to a specific crime scene situation where an association with a particular plant can be demonstrated, either through the case circumstances or by the finding of a dominant pollen type in the recovered assemblage resulting from direct transfer of pollen. An example would be the aforementioned case where the offender was seen to brush against a *Hypericum* plant upon leaving the scene of a sexual assault. However, depending on the diversity of the DNA within a species, there may not be any enhanced evidential significance beyond the already striking significance of the simple presence of the peculiar pollen types on the clothing. A further complication in comparing pollen DNA to plant DNA is that as the pollen grain is the male gamete, it contains only one half of the DNA of the parent plant.

The hardy nature of pollen grains presents an issue for DNA profiling of assemblages present in soils (40). Although techniques exist that utilize their rugged properties to destroy everything else in the sample except the pollen wall, these processes are destructive towards the pollen grain contents and their DNA. Therefore, standard palynological techniques involving potassium hydroxide digestion, acid treatment, or acetolysis (12), cannot be utilized in the clean-up steps prior to DNA analysis. Therefore, more passive techniques such as density-gradient separation are required, followed by a "gentle" mechanical disruption of the pollen wall to release the cellular contents (40). It is also important to remove from the sample any substances that may inhibit the DNA amplification steps. Soil potentially contains many inhibitors such as humic acids that need to be removed during the clean-up procedure. Therefore, for effective DNA analysis of pollen concentrates from soil, it is essential to ensure that a contaminant-free concentrate is obtained. As some plant material, other than pollen grains, may have the same specific gravity as pollen, this extraneous plant material may be present in the concentrate. Therefore, as well as removing inhibiting substances from the extract, a simple check of the concentrate for extraneous plant material is also needed. The challenge for the future therefore is likely not to be whether or not DNA analysis techniques will be applied to forensic palynology, but rather how well developed the science of forensic palynology will be to support the work involving this important trace evidence type.

Conclusions

The application of palynology to forensic science could be judged as having had mixed success. There are many anecdotal stories where pollen evidence has had spectacular successes, as either investigative or associative evidence. On the other hand, this evidence type is extremely underutilized in most countries. Furthermore, the unstructured way in which the science has been applied to forensic problems has led to a lack of formalized discussion of the scientific principles underlying the applications, with little experimentation and modeling to show validity.

In the current legal environment, where there is renewed questioning of the acceptability of most evidence types, it would be prudent for forensic palynologists to lead the establishment of palynological evidence through validation-type studies and experimentation. Through this a solid foundation can be laid, upon which, with renewed promotion of the science and greater understanding of the significance of the evidence attained, forensic palynology can move towards greater acceptance internationally, rather than being a much-vaunted tool for rather localized jurisdictions.

References

1. Locard E. The analysis of dust traces (second part). Am J Police Sci 1930;1:401–18.
2. Erdtman G. Handbook of palynology. New York: Hafner Publishing Co., 1969.
3. Stanley EA. Forensic palynology. Federal Bureau of Investigation International Symposium on Trace Evidence. Washington, DC: Government Printing Office, 1993.
4. Bryant VM, Mildenhall DC. Forensic palynology: a new way to catch crooks. In: Bryant VM, Wrenn JW, editors. New developments in palynomorph sampling, extraction, and analysis. Dallas, TX: American Association of Stratigraphic Palynologists Foundation. Contributions Series Number 33, 1998;145–55.
5. Mildenhall DC. Forensic palynology in New Zealand. Rev Palaeobot Palynol 1990;64:227–34.
6. Eyring MB. Soil pollen analysis from a forensic point of view. Microscope 1996;44:81–97.
7. Horrocks M. Sub-sampling and preparing forensic samples for pollen analysis. J Forensic Sci 2004;49:1024–7.
8. Milne LA, Bryant VM, Mildenhall DC. Forensic palynology. In: Coyle HM, editor. Forensic botany. Washington DC: CRC Press, 2005;217–52.
9. Kirk PL. Crime investigation. New York: Interscience Publishers, 1953.
10. Inman K, Rudin N. Principles and practice of criminalistics. The profession of forensic science. Boca Raton, FL: CRC Press, 2000.
11. Faegri K, Iversen J. Textbook of pollen analysis, 4th ed. Chichester: John Wiley & Sons, 1989.
12. Moore PD, Webb JA, Collinson ME. Pollen analysis, 2nd ed. London: Blackwell Scientific, 1991.
13. Tauber H. Differential pollen dispersion and filtration. In: Cushing E, Wright H, editors. Quaternary paleoecology. New Haven: Yale University Press, 1967.
14. Graham A. Forensic palynology and the Ruidoso, New Mexico plane crash—the pollen evidence II. J Forensic Sci 1997;42:391–3.
15. Lewis WH. Pollen composition in a crashed plane's engine. J Forensic Sci 1997;42:387–90.
16. Mildenhall DC. An example of the use of forensic palynology in assessing an alibi. J Forensic Sci 2004;49:1–5.
17. Mildenhall DC. *Hypericum* pollen determines the presence of burglars at the scene of a crime: an example of forensic palynology. Forensic Sci Int 2006;163:231–5.
18. Horrocks M, Walsh KAJ. Fine resolution of pollen patterns in limited space: differentiating a crime scene and alibi scene seven meters apart. J Forensic Sci 1999;44:417–20.
19. Horrocks M, Coulson SA, Walsh KAJ. Forensic palynology: variation in the pollen content of soil surface samples. J Forensic Sci 1998;43:320–3.
20. Horrocks M, Coulson SA, Walsh KAJ. Forensic palynology: variation in the pollen content of soil on shoes and in shoeprints in soil. J Forensic Sci 1999;44:119–22.
21. Frye v United States, 293 F. 1013 (D.C. Civ 1923).
22. Daubert v Merrell Dow Pharmaceuticals Inc., 113 S. CT. 2786 (1993).
23. Peterson JL, Markham P. Crime laboratory proficiency testing results, 1978–1991, I: identification and classification of physical evidence. J Forensic Sci 1995;40:994–1008.
24. Peterson JL, Markham P. Crime laboratory proficiency testing results, 1978–1991, II: identification and classification of physical evidence. J Forensic Sci 1995;40:1009–29.
25. Randerson J. Grassed up. New Sci 2002;2332:32–5.
26. Mildenhall DC. Forensic palynology. Geol Surv N Z Newsl 1982;58:25.
27. Moran B, Murdock J. Joseph Rameriz vs. State of Florida—Supreme Court Decision December 20, 2001. AFTE Journal 2002;34:215–26.
28. Nichols RG. Firearm and toolmark identification criteria: a review of the literature. J Forensic Sci 1997;42:466–74.
29. Grzybowski RA, Murdock JE. Firearm and toolmark identification—meeting the Daubert challenge. AFTE Journal 1998;30:3–14.
30. Palenik S. Dust. In: Siegel JA, Saukko PJ, Knupfer GC, editors. Encyclopedia of forensic sciences. London: Academic Press, 2000;662–7.
31. Horrocks M, Walsh KAJ. Forensic palynology: assessing the value of the evidence. Rev Palaeobot Palynol 1998;103:69–74.
32. Bruce RG, Dettman ME. Palynological analyses of Australian surface soils and their potential in forensic science. Forensic Sci Int 1996;81:77–94.
33. Horrocks M, Ogden J. An assessment of linear discriminant function analysis as a method of interpreting fossil pollen assemblages. N Z J Bot 2003;41:293–9.
34. Hutter T. Palynology: a new tool for the forensic investigator. In: Godwin GM, editor. Criminal psychology and forensic technology. A collaborative approach to effective profiling. Boca Raton, FL: CRC Press, 2001;15–28.
35. Treloar WJ, Flenley JR, Empson L. Towards automation of palynology 2: the use of texture measures and neural network analysis for automated identification of optical images of pollen grains. J Quaternary Sci 2004;19:755–62.
36. Treloar WJ, Taylor GE, Flenley JR. Towards automation of palynology 1: analysis of pollen shape and ornamentation using simple geometric measures, derived from scanning electron microscope images. J Quaternary Sci 2004;19:745–54.
37. Simel EJ, Saidak LR, Tuskan GA. Method of extracting genomic DNA from non-germinated gymnosperm and angiosperm pollen. Biotechniques 1997;22:390–4.
38. Zhou L-J, Pei K-Q, Zhou B, Ma K-P. A molecular approach to species identification of Chenopodiaceae pollen grains in surface soil. Am J Bot 2007;94:477–81.
39. Ferraby HL. Application of DNA technology to pollen analysis (MSc thesis). Auckland: University of Auckland, 2004.
40. Eliet JR. The development of a DNA analysis system for pollen (MSc thesis). Auckland: University of Auckland, 2005.

Additional information and reprint requests:
Mark Horrocks, Ph.D.
Microfossil Research Ltd.
31 Mont Le Grand Rd
Mt Eden, Auckland 1024
New Zealand
E-mail: info@microfossilresearch.com