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Forensic Ecology, Botany, and Palynology: Some Aspects of Their Role in Criminal Investigation

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Abstract Ecology, botany, and palynology are now accepted as part of the armoury of forensic techniques. These disciplines have been tested in court and have provided evidence for contact of objects and places, location of clandestinely-disposed human remains and graves, estimating times of deposition of bodies, differentiating murder sites from deposition sites, and provenancing the origin of objects and materials. It is important that the forensic palynologist is a competent botanist and ecologist. Sadly, not all practitioners have this essential background and, therefore, produce work inadequate to withstand scrutiny in court. Palynology involves the identification of many classes of microscopic entities, the most important being pollen, plant spores, and fungal spores. The practitioner needs to be able to identify palynomorphs in damaged and decayed states and this requires experience and skill. However, identification is still the lowest level of palynological expertise, and interpretation of palynological assemblages requires knowledge of plant distribution, developmental responses, and phenology, as well as ecosystem structure and function. The forensic palynologist must also understand highly manipulated and artificial systems, and the complexities of taphonomic processes. There have been attempts to make forensic palynology 'more scientific' by the construction of test trials, the application of current statistical techniques, mathematical modelling, and reference to aerobiological data and pollen calendars. But these appear to be of limited use in the forensic context where outcomes are scrutinised in court. There is a high degree of heterogeneity and variability in palynological profiles, and every location is unique. It is impossible to achieve meaningful and forensicallyuseful databases of the palynological characteristics of places; predictive models will always be crude and unlikely to be of practical value. In spite of this, the experienced ecologist/palynologist has been able to identify places, demonstrate links between objects and places, estimate body deposition times, and differentiate pertinent from irrelevant places very successfully. Nevertheless, there has been no substitute for examination of every pertinent place, and every relevant exhibit in each criminal investigation.

Keywords: Forensic ecology, Botany, Palynology, Criminal investigation, Forensic Science.

Introduction

Ecology is the study of organisms together with their environments – the study of ecosystems. By its nature, palynology is a subdiscipline of botanical ecology and, to work in a forensic context, the palynologist must have a sound botanical and ecological training. In Britain, forensic palynology is an acknowledged aid to criminal investigation, providing valuable evidence in cases of murder, manslaughter, rape, and abduction. The body of literature for the discipline in peer-reviewed journals is relatively small and, although many reports and interpretive material are technically in the public domain, they are only accessible through court and police records.

The crime scenes which benefit from palynological help are invariably ecosystems themselves but they may be highly modified by human activities. Therefore, as well as the understanding of natural and semi-natural habitats, the forensic ecologist/palynologist must also appreciate the complexities of highly manipulated systems, such as gardens, parks, rubbish dumps, plantations, ponds, canals, roadsides verges, hedgerows and wasteland. Because of the breadth of the discipline, the forensic ecologist cannot be expert in every aspect of ecological science but, to be of use to a criminal investigator, the essential requirements are knowledge of soil, and of aquatic and terrestrial sediments.

Soils and sediments exhibit great variability in the origin of their parent materials, structure, and chemistry but it is important for the forensic ecologist and palynologist to realise that soil is particularly complex because of its dynamic nature. It provides a habitat where communities of organisms live and complete their life-cycles, and these organisms profoundly affect the chemistry of the inorganic matrix as well as any organic object or material present.

Most plants rely on soil as a

source of mineral nutrition, water, and physical support. Depending on their responses to climate, microclimate, and their ecological tolerances and needs, the geographical distribution of plant species can reflect historical geography, and the patterning of soil types at local, regional, and national levels. Plant distribution is also profoundly affected by biotic factors – other plants, animals, micro-organisms and people. Again, the forensic ecologist and botanist must have an understanding of the factors underlying plant distribution, plant response to change, and to have a grasp of the variability created by human intervention. This is achieved by strengthening and modifying theoretical knowledge with extensive field experience.

Over time, organic components of soil will decompose to their constituent molecules. The speed of decomposition will depend on the communities of resident decomposer organisms, and their function depends largely on the physico-chemical nature of the soil itself. Palynomorphs are important organic particles in soils and sediments and, in recent years, these have provided valuable trace evidence in criminal investigation. Originally, the term ‘palynomorph’ was used to describe pollen grains and plant spores. Over the years, however, the term has expanded to include: other microscopic plant remains such as trichomes (plant hairs and glands); fungal spores and other fungal bodies; diatoms; cyanobacteria; and microscopic animals such as testate amoebae, nematode eggs and mouth parts, mites, and other arthropod body parts. The palynologist needs to be able to identify many of these kinds of palynomorph, or seek additional expertise.

Applications of Ecology, Botany, and Palynology in Criminal Investigation

I have contributed ecological, botanical, and palynological evidence in over 200 criminal cases, and presented it for cross examination in court on many occasions. Table 9.1 lists a range of objects and matrices from which I have analysed thousands of samples (see also Milne et al. 2005). There are many cases where ecology, botany, and palynology have successfully helped in: (i) linking objects and places (e.g. Wiltshire 2006a; Mildenhall, 2006); (ii) locating hidden human remains and provenancing of objects (Brown et al. 2002; Wiltshire 2005a); (iii) estimating temporal aspects of deposition of remains (Szibor et al. 1998; Wiltshire 2002a; 2003b); and, (iv) differentiating murder scenes from deposition sites (Wiltshire 2002b).

Knowledge of the anatomy of plants, animals, and other organisms helps in the identification of what victims have eaten or inhaled before death, and whether or not an object is of biological rather than manufactured origin (Wiltshire 2003a, 2004a; 2006b). An understanding of plant and fungal development, and the activity of scavenging animals, has given valuable information on the length of time a corpse has lain in situ or the length of time since an offender walked on vegetation (Hawksworth 2008a; Wiltshire 2007a, b). Knowledge of soil stratigraphy, coupled with plant development and distribution, has resulted in establishing the premeditated nature of a victim’s grave (Wiltshire 2005b). Finally, exploitation of knowledge of plant and fungal taxonomy (Hawksworth 2008b; Wiltshire 2005c, 2008) has been used in assessing the potential of plants being involved in attempted murder by poisoning, or manslaughter through shamanism.

Therefore, it is clear that the identity, structure, chemistry, life-cycles, and growth responses of whole organisms play an important role in criminal investigation, and that even fragments of organisms provide valuable forensic evidence.

Palynology: The Background

The study of palynomorphs gave rise to the science of palynology, first coined by Hyde and Williams in the 1940s (Hyde and Williams 1944). Its derivation is from the Greek verb palynein, meaning ‘to spread or sprinkle around’. Hyde and Williams were aeropalynologists, concerned with airborne allergens, and had little interest in soil palynomorphs. Their work involved trapping airborne particles and identifying temporal sequences of anthesis (pollen release) for the construction of ‘pollen calendars’.

The discipline of palynology is now over 100 years old. The founder of modern pollen analysis was Swedish geologist, Lennart von Post, but the subject was developed and promoted by fellow Swedish botanists Rutger Sernander and Gustaf Lagerheim. The first major work in the subject was published by Erdtman (1921).

The first recorded cases of palynology being used as a forensic tool were described by Erdtman (1969). Although applied occasionally (e.g. Frei 1979; Nowicke and Meselson 1984), it has only been used more routinely in the last 15 years or so. Mildenhall pioneered the techniques in New Zealand, Bryant in the United States (Mildenhall 1982; Bryant et al. 1990; Bryant and Mildenhall 1998), and I have developed forensic palynology, forensic ecology, and forensic botany in the British Isles.

Palynomorphs and Their Identification

The range of palynomorphs requiring identification in criminal investigation has grown, and every attempt should be made to identify anything of apparent significance in a palynological preparation. That said, the most abundant and frequently encountered palynomorphs are pollen grains and plant spores. There are many web sites and publications providing pictures, diagrams, and descriptions of pollen and spore structures to facilitate their identification (e.g. Moore et al.

1991; Beug 2004). However, there is no substitute for authenticated reference material. Any unknown pollen grain or spore must be compared with actual, accurately identified material and every attempt made to obtain a prepared or fresh specimen. Serious misidentifications have been made by those relying solely on pictures. While this is regrettable in any area of palynological study, it could have dire consequences in forensic investigation. Keys and pictures should only be used as guides; final identification must involve critical comparative examination of actual palynomorphs under the microscope.

Pollen and plant spores are identified by their shape, size, outer wall (exine) structure, surface sculpturing, and the type, number, and arrangement of apertures. To achieve the highest resolution of identification, it is essential to remove the inner part of the grain so that only the exine remains, involving use of toxic and corrosive acids. It results in the dissolution of background humic material, cellulose, and silica, and only structures which are resistant to the treatment will be retained (Moore et al. 1991). These include the outer walls of pollen and plants spores which are composed of sporopollenin, a very robust polymer. Fungal and arthropod remains, composed of chitin, and some testate amoebae, will also be left after treatment. Although not so important for the identification of some non-botanical palynomorphs, the chemical processing of pollen and plant spores is critical for precise identification. Some sculpturing features of the palynomorph are small (e.g. 0.5 μm), needing observation under phase contrast microscopy at ×1000 or more.

The resolution in pollen and spore identification is variable. In some plant families, taxa can be identified to species (e.g. Plantago lanceolata – ribwort plantain; Sanguisorba officinalis – greater burnet; Centaurea scabiosa – greater knapweed). Others can only be identified reliably to genus (e.g. Quercus – oaks; Salix – willows; Polygala – milkworts; Aesculus – horse chestnuts), while some can only be identified to family (Cupressaceae – cypress; Chenopodiaceae/Amaranthaceae – goosefoot family; Poaceae – grasses). In some families such as the Rosaceae, taxa can be identified to species (Rubus chamaemorus – cloudberry; Agrimonia eupatorium – agrimony), to genus (Geum – wood avens and water avens), to type (Potentilla – type which includes three genera), and to groups of genera and species whose morphologies merge one into another and so are difficult to differentiate reliably (e.g. many Prunus – cherry/plum/peach/almond species). It is also difficult to differentiate between pollen taxa such as Rosa (roses), Rubus (brambles and others), Sorbus (rowan and others), Crataegus (hawthorns), and some species of Prunus are in the rose-bramble-hawthorn group. As introduced garden species and cultivars are important in the forensic context, particular care must be taken in identification of these groups, but they can also provide surprisingly distinctive markers.

Identification will be relatively crude in the absence of chemical processing. If the inner part of the pollen grain, and the soil matrix (or other material), are not removed from the background, only identification to family may be possible. Another source of error is in the identification of taxa which are impossible to differentiate by standard techniques. Knowing what is possible requires considerable experience of pollen and spores from of a wide range of species. Reference to text books will not always resolve the problem of inter-generic and inter-species variation. Further, many fossil spores (from the Mesozoic to Caenozoic eras), and other remains, find their way into palynological preparations; and these have been known to be wrongly identified as modern taxa. This is particularly the case for Pteridophyta (ferns and allies), sometimes identified as Sphagnum moss spores, or as modern fern spores. Fossil fungal remains are also retrieved from exhibits and considered modern. Fungal spores have been misidentified as pollen!

Many areas of palynological investigation (e.g. palaeoecology, melissopalynology) can achieve clean samples with palynomorphs in good condition (Figure 9.1a), but forensic palynology often involves examination of very poor samples (Figure 9.1b). The samples may be laden with cellulosic debris, fly-ash, soot, and other materials which can obscure the view of the palynomorph on the slide. The palynomorphs themselves might also be crushed, crumpled, broken, and partially decayed. In the case of forensic samples, there must be no positive opinion given unless the analyst can demonstrate criteria for identification which are robust enough for legal challenge.

Scanning electron microscopy (SEM) and other sophisticated microscopical methods (e.g. confocal electron microscopy) have little practical application for routine forensic palynological work. Although, on occasion, a sample might consist of a single taxon, identified by SEM, this is rare. SEM presents a picture of only the outer surface of the pollen grain or spore. In the case of pollen, it is often the elements making up the outer wall (exine) seen in section, that are pivotal for precise identification. These details are more easily differentiated by high-powered, bright field and phase contrast microscopy. In forensic samples, it is usually necessary to identify and count hundreds of palynomorphs found in a prepared slide and, although it is technically feasible using SEM (Jones and Bryant 2007), the experienced palynologist does not need SEM for identification. If only SEM micrographs are available, identification can be impossible on some occasions.

Palynomorphs as Trace Evidence

Locard's Principle ('every contact leaves a trace') is known to every police detective (White 2004). As outlined above, palynomorphs, especially pollen and spores, are excellent proxy indicators of place. Offenders walk on soil, mud, or vegetation (short and tall); they have been known to hide in, or walk through, hedges, lean against buildings, trees, and posts, or sit on seats. Important evidence has been retrieved from very many objects and matrices and some of these are shown in Table 9.1. If palynomorphs are transferred from a place to an offender, a victim, or any object, they can be retrieved. The transferred assemblage can then be evaluated in terms of the likelihood of the offender, or victim, or object having contacted the specific place (Wiltshire 2004c).

Pollen grains have evolved for sticking to the female part of the plant and, unlike fibres (which are readily shed from clothing and other objects), will embed into fabrics and small interstices in footwear and other objects; pollen and spores are not easily removed. They are held firmly by their surface sculpturing and by static charges, and are not easily shed, even from clothing and footwear that have been subjected to washing in a machine (Wiltshire 1997). This quality of tenacious adherence makes them very valuable as trace evidence and indicators of places or specific surfaces. The value and advantages of palynology to forensic investigation are obvious and the discipline proven to be effective. However, it is not simple and the practitioner may need to apply caveats to any conclusion.

Caveats and Limitations

It is important to be aware of caveats and limitations in forensic palynology as the outcome of the work could result in someone losing their liberty. It is not an academic exercise. All environments are highly variable, particularly those outside buildings. Even run-down inner-city estates can have surfaces yielding distinctive palynological profiles with areas of bare soil, lawns, weedy cracks in pavements and corners, and some vegetation. But great care is required in planning the sampling strategy, the refinement of preparation, the resolution of palynomorph identification, and the interpretation of the volume of data gathered.

One of the most commonly-used arguments against the expert witness palynologist is that the observed profile could have been picked up from 'anywhere'. Another common challenge is that the observed profile had accumulated through repeated contact with a variety of palyniferous surfaces, each contributing to a profile that, collectively, happens to be characteristic of the crime scene. The reply would need to stress the improbability of this happening. Experience has shown that, while every place will yield a unique profile, some places are similar to others in various degrees. Considerable credibility as to the uniqueness of an assemblage is achieved when palynomorphs that are generally rare (in palynological terms) are found in the profile.

Databases and Statistical Analyses

It might be considered that a reference database could be compiled of palynological profiles of oak woodlands, grasslands, roadside verges, or any other kind of recognisable environment. This might be feasible in the broadest sense but only at a level where the resolution would never be sufficiently high to be of value in criminal investigation. Databases for populations of palynomorph species can be constructed but not for assemblages from places.

This can be exemplified by Hampshire murder case (Wiltshire and Black 2006) where a man was garrotted beneath a beech tree (Fagus sylvatica) and placed, face-down, in a shallow grave immediately adjacent to where he was killed. The area around the grave also had holly (Ilex aquifolium), oak (Quercus robur), birch (Betula pendula), pine (Pinus), honeysuckle (Lonicera periclymenum), and bramble (Rubus fruticosus), with a ground flora dominated by, amongst others, bluebell (Hyacinthoides non-scripta), wind-flower (Anemone nemorosa), and bracken (Pteridium aquilinum). The palynomorph assemblage in the comparator samples was complex; many other taxa (some relatively uncommon in palynological assemblages) were recorded in addition to the most obvious plants growing in the vicinity of the grave. The assemblages retrieved from footwear and a vehicle belonging to two suspects were very similar to that in the comparator samples from the crime scene. Included in the assemblage were abundant conidia (asexual spores) of Triposporium elegans. Although found on a range of woody species, this fungus is very common on the cupules of beech fruits.

An inevitable argument likely to have been presented by Defence Counsel, and which needed addressing, was that the assemblage could have 'come from any woodland in Hampshire and Sussex'. Investigation revealed 14 woodlands that could, conceivably have had the same species composition as the crime scene. Each was extensively field-walked with the purpose of finding an environment which might offer a similar palynological assemblage to that from the crime scene. Three were found, sampled, and analysed. None resembled the crime scene closely, although all the dominant species were growing in the three places. Furthermore, only one yielded Triposporium elegans, even though all three were thickly strewn with beech cupules; this fungal spore helped eliminate two of the sites.

Pollen and spore reference material in a good collection will contain examples taken from different anthers/sporangia, different plants, and different places at different times for each species; and to be competent, a palynologist requires access to an authenticated and comprehensive reference collection of pollen, plant spores, fungal spores, and other microscopic entities. Although there have been occasions where a single palynomorph taxon has been useful in a criminal case (Mildenhall 2006), it is rare for one palynomorph or single palynomorph taxon to be of evidential value. More usually, the evidence consists of palynological assemblages comprised of up to 200 or more different palynomorphs in varying abundances. For many classes of trace evidence (e.g. fibres, glass, brick, paper, paint, and ink), although each is huge, their populations are finite; given time and resources, comprehensive reference collections for these types of evidence could be constructed for statistical comparison. To date this has proved impossible for whole palynological profiles.

Some attempt has been made to invoke Bayes' theorem to palynological data (Horrocks and Walsh 1998). Unfortunately, there were some false assumptions in their study, and the 'cases' appeared to be highly theoretical, simple, scenarios constructed to demonstrate their hypotheses. If full datasets from real cases had been used, the conclusions drawn may have been different. The Bayesian approach is important philosophically and is useful for framing the presentation of conclusions but, at the present time, it is difficult to see how it can be applied to the volume of data accrued from real cases. Multivariate analysis has, on occasion, proved useful in dealing with data where there are high numbers of exhibits and comparator samples (Wiltshire 2002c, 2004a; Riding et al. 2007), but only for guidance. Conclusions, and professional opinion presented in court, must be based on botanical and palynological criteria, skill, and experience.

Taphonomy may be defined as 'The sum of all the factors that influence whether a palynomorph (pollen, spore, or other microscopic entity) will be found at a specific place at a specific time' (Wiltshire 2006a). The taphonomic processes influencing the pattern of palynomorph deposition in any environment are numerous. Unlike populations of fibres, glass and other man-made materials, those of palynological profiles are infinite. The many variables that affect the accumulation of palynomorphs in soils, on surfaces, and on items such as footwear, vehicles, and clothing, preclude routine statistical techniques from making useful contributions to forensic palynological studies.

If two sampling points are spatially close, there will be a higher likelihood of them being similar than if they were widely separated. Similarity diminishes with distance although, under some circumstances, samples in close proximity to each other can be distinctly different. My interpretation of many thousands of samples has shown that every place will yield a unique palynological profile, and that even each sampling point within a site will have its own special characteristics. To gain a palynological 'picture of place' requires the analysis of many comparator samples to build up a matrix of profiles, each one contributing to the bigger picture. Such a bigger picture will be unique for that place.

The complexity of palynological taphonomy makes the discipline an exquisite tool in the hands of the experienced forensic palynologist. However, it can be potentially misleading and suspect when those with inappropriate, or insufficient, experience are involved, and the caveats and constraints are not properly addressed.

Taphonomic Considerations

Some of the major taphonomic factors affecting palynological profiles have been briefly reviewed by Mildenhall et al. (2006). They note that of importance is the level of pollen and spore production, and the way these entities are dispersed.

A spore produced by a fungus, moss, or fern will germinate and form a new organism if conditions are amenable. Pollen produced by conifers and flowering plants is carried to female stigmas to effect fertilisation and production of seeds. Both spores and pollen are carried by vectors, mostly wind, insects, and rain-splash. Those pollen and spores, which achieve dispersal into turbulent air and get carried up and away from the parent plant, form the 'airspora'; this will eventually fall as 'pollen rain' onto surfaces.

In general, wind-pollinated plants produce large amounts of well-dispersed pollen, while insect-pollinated ones produce relatively small amounts of poorly-dispersed pollen. Pollen derived from wind-pollinated trees and shrubs, and tall, wind-pollinated herbs are common components in the airspora, and are often over-represented. But crime scenes are often dominated by insect- or self-pollinated plants. Invariably their pollen simply falls in a halo on the ground around the parent plant, or is released only when the plant dies and falls to the ground. Pollen from these plants, as well as many fungal spores (including those of lichens), may never be found in the airspora, and contribute little to the pollen rain, but they may be the most abundant on an item of footwear. The wearer may have walked through organic debris or meadow vegetation, and abundant pollen from the airspora at the time of the offence may form only a small proportion of the profile relevant to the criminal investigation.

Some palynomorph taxa are thus exceptionally useful markers of the place and, if there were many such plants at a specific location, that place would be endowed with a characteristic palynological signature which would be difficult to replicate artificially. Analysis of many samples at numerous crime scenes has demonstrated that whatever the mode of pollination, the major part of the pollen load of any plant falls close to the parent.

Many palynologists are engaged in the reconstruction of past environments, vegetation change through time, and past land-use; this involves the analysis of cores of mire or lake sediments, or buried soils. Few carry out independent and extensive analysis of multiple surface samples at either local or regional level. Most depend on the researches of other palynologists interested in the taphonomic problems associated with dispersal and fall-out. The latter includes the Pollen Monitoring Programme, a research initiative sponsored by the International Quaternary Association (INQUA) (Hicks et al., 2001; Tinsley, 2001; Barkenow et al., 2007). Another initiative, POLLANDCAL (Pollen-Landuse Calibrations), involves many palynologists collecting modern pollen data and using sophisticated statistical techniques to generate predictive models (Sugita et al., 1999; Eklöf et al., 2004; Bunting et al., 2005; Bunting and Middleton, 2005; Soepboer et al., 2007).

As with earlier studies on pollen productivity and dispersal, the work of the PMP and POLLANDCAL is, in the main, concerned with obtaining information to enhance interpretation of palaeoecological profiles. It aims to interpret past vegetation by extrapolation of results from patterns of modern pollen deposition to profiles obtained from ancient deposits.

Airspora studies depend largely on the use of pollen traps (see Caulton et al. 1995; Levetin et al. 2000; Wiltshire 2006a). Although there are many trapping sites, the areas covered are, nevertheless, insignificant in comparison to the total land surface available for deposition. Extrapolating modern data to the past requires a leap of faith since there may be no past homologue or analogue of modern vegetation patterning. The situation is less tenable when data from pollen traps are applied to more distant sites, and inappropriate when applied to ancient sites, hundreds of miles from the sampling locations. Irrespective of the sophistication of statistical modelling, the nature of the pollen trapping site is critical. There may be common elements in vegetation composition between the sampling site and a palaeoecological profile, but the chance of them having been truly representative of the ecology of another is remote.

The study of airborne palynomorphs is fraught with difficulty and has been a focus of debate for many years. There is considerable variation in the pollen rain at any one place at any one time, and this variation is reflected in fall-out patterns onto surfaces. The variation will depend on pollen production and dispersal characteristics, the presence of physical and geographical barriers, the structure and mass of the palynomorph affecting sedimentation rates, and many other factors. There are classic models constructed to explain the observed heterogeneity in the airspora (Tauber 1965, 1967), and large-scale and small-scale modern pollen studies which have led to the construction of predictive models, often quoted by palynologists working on the reconstruction of past vegetation and land-use (Davies 1967; Jacobson and Bradshaw 1981; Prentice 1985).

A search on the internet provides large numbers of papers, written over the last 40 to 50 years or so, describing and examining these taphonomic phenomena, as well as more recent research projects and papers. There is little doubt that data collected through the PMP and POLLANDCAL, and specific studies involving dispersal from individual species, are not sufficiently refined for use in forensic studies. Inevitably, their investigations concentrate either on palynomorphs, which become airborne and contribute to the airspora, or are geared towards plant/vector inter-relationships. The former are mostly trees, shrubs, and wind-pollinated herbs which ave high pollen production and good dispersal, and the latter related to crop plants which rely on insect pollination.

There is some danger in adhering to some of the conventional wisdoms in palynology where models are based on the work of a limited number of researchers in a limited range of scenarios. For example, it is often assumed that some taxa such as Pinus (pine) exhibit long distance transport. Some grains are capable of being transported many miles from source but, if there are physical obstacles between the source and accumulating surface, the pollen may be deposited only very locally. In every plant, most of its pollen or spore production will fall near the parent. Enigmatically, there have been cases where a prolific pollen producer such as pine registered less than 2% of the total pollen sum even though mature trees were within 10 m of the sampling site (Wiltshire unpubl.). In other instances, insectpollinated plants such as Aesculus hippocastanum (horse chestnut), where pollen production is thought to be low and dispersal poor, have achieved the same values as Quercus (oak), a more prolific pollen producer, several hundred metres away from a mixed stand of horse chestnut and oak in a public park (Wiltshire unpubl.).

At a crime scene in Brierley Hill, near Birmingham, several samples from the vicinity of a young mature, fruiting tree of Fagus sylvatica (beech) failed to yield any beech pollen (Wiltshire 2003c). A similar situation was observed in a case in South Wales (Wiltshire 2004c) where a murder victim was buried on a hillside, dominated by Picea sitchensis (sitka spruce). Analysis of the surface soils around the grave showed that spruce pollen hardly registered in the comparator samples, but that pine pollen was very well represented. In the lower fill of the grave-fill, the assemblage was dominated by pine. The spruce trees were very large and it might be expected that their pollen would swamp the surface. However, very few of the trees had reached sexual maturity. A single, small but mature, pine tree about 100 m from the site was the source of the surface pine pollen. That in the deeper profile was enigmatic, but could have represented the vegetation before the spruce was planted about 40 years earlier. Without understanding such systems, or by not visiting the crime scene, the palynologist might well have assumed pine rather than spruce woodland to be associated with the suspects.

These few examples demonstrate the danger of adhering strictly to simplistic scenarios. Further examples of the dangers of relying on airspora data in forensic investigation are found in Wiltshire (2006a).

Source of Trace Evidence

Pollen and spores falling at any one time will be mixed with pollen previously accumulated on the surfaces. Plants (both insect and wind-pollinated) colonising new ground will also contribute to pre-existing assemblages. This means that time is important in forensic sampling. A natural/semi-natural habitat such as a woodland might yield very similar profiles for many years, but there could be drastic changes if the environment were a manipulated one, such as a plantation or garden, even within short periods. Further, any object contacting a palyniferous surface will receive only a fragment of the pollen rain that had accumulated on it over time, and a fragment of the biological signature of the habitat as a whole. This is why it is essential for the palynologist to select target locations within a crime scene.

Although trace evidence is transferred to the belongings of offenders when they contact soil and sediments, there have been cases where soil has not played any role in investigations (Wiltshire 1997, 2007b). Many surfaces are completely vegetated, or covered in deep leaf litter, such that soil may not be contacted by footwear, clothing, or tools, and vehicles. Plant surfaces, plant litter, humus, and compost can yield dust and, perhaps, the fine fraction of the soil through rain splash and wind action, but the most important particulates will be biological. The investigator must be aware that footwear, the outside of vehicles, and digging implements might be irrelevant to a case and the main source of evidence would be the clothing on the upper body, with no trace of soil.

The palynological profile from any crime scene is built up from multiples of comparator samples; it is, therefore, composed of a pattern of fragments. It also follows that, to gain a workable picture of the place, the larger the sampling area, and the greater the number of samples obtained, the closer the results will be to the actual profile (even though that is unknowable in detail). An offender contacting a crime scene will pick up only a fragment of the crime scene's palynological profile. If the trace evidence is then secondarily transferred to, say, a vehicle, only a thirdorder fragment will be retrieved. Palynological interpretation is, therefore, complex and requires visualisation skills as well as an understanding of the complex taphonomy underlying assemblages.

In spite of all the caveats that apply, the assemblages distinctive enough to establish convincing links between items, places, and vehicles have been repeatedly demonstrated. As previously stated, palynological samples obtained from exhibits are fragmentary in nature. For links between them and crime scenes to be acceptable to the Court, there needs to be either: (a) a highly complex assemblage where there are many points of similarity between place and object, or (b) some unusual or rare component or components.

Mixed Samples: Fabrics

Garments worn repeatedly for considerable periods will pick up palynomorphs from various places, so any retrieved assemblage will be mixed. They are transferred easily from palyniferous surfaces, but few seem to be picked up from air. Except where there is obvious soiling, it is impossible to separate various depositional events by sampling. But, unlike footwear, most items of clothing generally have limited contact with soil, vegetation, and other intensely palyniferous surfaces. As with footwear and vehicles, sufficient comparator samples are needed to be able to eliminate sources other than the crime scene but, if the assemblage accumulated from the crime scene is sufficiently distinctive, multiple deposition need not be an insurmountable problem.

A complication with fabric is that an offender may already have had soil on clothing before committing the offence, or after visiting the burial site. Palynomorphs from the crime scene can then be superimposed on the pre-existing soil marks. In one case there was an apparent conflict of evidence where a soil scientist and palynologist were not aware of each others' roles (Wiltshire 2001b). Soil on the suspect's sweatshirt was 'innocent', and was derived from deep sub-soil accumulated during the digging of arage foundations; analysis of the soil from the excavation showed it to contain no palynomorphs. While wearing the soiled clothing, the offender buried the victim near a hedge in a pasture. Before the grave was dug, there was little exposed soil in the meadow but, importantly, the offender picked up spores and pollen from tall vegetation on the path to and from the area around the grave site on his soiled sweatshirt. The palynomorph assemblage on the garment was similar to that at and around the deposition site. Thus, if only the soil evidence had been taken, there would have been no link between the garment and the burial site. This case provided a salutary lesson to investigators; the soil analyst and palynologist should work together to gain the deepest level of understanding from the respective data.

It is one of the strengths of palynology that pollen grains and plant spores will embed themselves in fabriucs such that they can be retrieved from exhibits even after being put through the washing machine (Wiltshire 1997).

Mixed Samples: Footwear

Footwear presents another

complex of problems and challenges. Usually, samples are taken from specific areas within the crime scene, known to have been walked upon by a suspect, so that they can be compared with palynological assemblages on the footwear. An offender will have had to contact the edge (and inside) of a grave during digging, and a rape victim might be able to locate the exact places trodden by her attacker; samples should be taken from such identified locations. Any footprints or depressions in soil and mud are obvious targets, but these are usually seized by the police for casting and foot mark analysis. It is now standard practice to scrape away the deposit at the interface of the underneath of the cast and the adhering soil layer to obtain the most relevant comparator sample. Even if the offender accumulated layers of soil/mud from elsewhere prior, or subsequent, to the offence, the mixed profile on the footwear should contain some of the trace evidence retrieved from the cast. The palynologist then has to differentiate the relevant profile from the irrelevant one.

There has been some attempt to make forensic palynology more 'scientific' by setting up hypothetical crime scenes and testing outcomes from various kinds of contact. Such studies are useful exercises and, for the objects used in the experiment, or places tested, the results might be valuable. However, some results presented, should be considered to be preliminary in view of the low pollen counts in each case, and the limited number of treatments within the trials (e.g. Riding et al. 2007). For different sets of footwear exposed to the same palyniferous surfaces, or other footwear exposed to other palyniferous surfaces, the outcomes might be very different. It is dangerous to formulate predictive models, or form firm conclusions, based on relatively few test items, in a few test scenarios, with low pollen counts. Footwear invariably accumulates multiple depositions of palynomorphs. It is, therefore, often necessary to count many hundreds (sometimes thousands) of pollen and spores to achieve an assemblage large enough to allow the differentiation of the crime scene from other places where palynomorphs may have been transferred to the footwear. If footwear yielded sparse palynomorphs, low counts might still be useful, but only if there were some very distinctive components present in the assemblage.

My analysis of thousands of items of footwear, including wellington boots, baby's bootees and Gucci court shoes, has shown that variation is so great that general models are unlikely to be attainable goals. There are many variables associated with the palyniferous material itself (soil, sediment, leaf litter, vegetation), but there are others which can affect the deposition and removal of palynomorphs. These include the materials making up the footwear, the gait and wear patterns of the wearer, the weight of the wearer, and even ambient weather conditions (and hence the wetness of the surface). For criminal investigation, in every case, it is important that microscopic analysis of footwear and other items is carried out so that they can be compared directly with comparator samples from the crime scene and other pertinent places. In the forensic context, a model is never likely to provide adequate information for prediction of events or outcomes.

By its very nature, at any one time, footwear will have a palynomorph load accumulated from a range of different places. Depending on the frequency and pattern of wear, trace evidence will continually be gained and lost. In my experience relatively few pollen grains are picked up from paved or metalled surfaces, although spores can be transferred from lichens growing on hard-standing. Palynomorphs can also be picked up from pavements and gravel paths on which decomposed and decomposing plant litter have accumulated. Invariably, the most significant assemblages of palynomorphs of all kinds are picked up from bare soil, mud, leaf litter, organic debris, and vegetation. Day-to-day, most people do not usually wear muddy or soiled shoes, and any noticeable accumulations of soil or mud on footwear are obvious targets for sampling in criminal investigations. These have proved useful in linking footwear with crime scenes and other sites but, more commonly, footwear from suspects in criminal cases is relatively clean or only slightly soiled. If there were several obvious depositions of soil/mud, then every attempt should be made to analyse each one separately. However, even when sampling is meticulous, it is rare for perfectly uncontaminated samples to be obtained; the palynological preparations will contain mixed assemblages.

In some cases, the pertinent layer of material on a shoe can be beneath subsequent, superficial layers of soil and mud, and it becomes impossible to differentiate them physically. Here, the situation is similar to that of the relatively clean shoe where the whole item must be sampled. There is no substitute for counting many hundreds of palynomorphs (or as many as possible) and comparing them with the crime scene as well as other pertinent places for elimination purposes.

A complicated case was that of R vs Anthia (Wiltshire 2004d) concurrently investigated by two police forces. Because of the modus operandi, the two forces suspected that the same man was responsible for at least six attacks in Hertfordshire and London. I visited the six crime scenes, which varied from woodland, roadside verge hedges, golf courses, and wooded areas of parks. I was given the suspect's clothing, items from his vehicle, and several items of footwear. There were convincing palynological similarities between one of the Hertfordshire sites and the upper clothing, car seat, and one pair of footwear. The palynology of another pair of boots yielded very similar assemblages to another Hertfordshire crime scene as well as one of the London sites. These two crime scenes were sufficiently distinctive that both could be recognised in the mixed assemblage on the boots. The defendant was convicted and received 10 life sentences. Approaching 20,000 palynomorphs were counted in this case, and it was the exceedingly high resolution of the analysis which allowed the various crime scenes to be differentiated.

It is now standard practice to analyse each item in a pair of footwear separately. In many cases, both feet pick up similar palynological assemblages and it may be thought unnecessary to do separate analysis. However, there have been at least two cases where each shoe differed, and the results were pivotal to interpretation of the cases. In a drugs-related case (Wiltshire 2001a), an informant claimed that, although he had stood on an area of hard-standing at the edge of a woodland where a grave had been dug, he had not entered the scene and remained standing on an area of muddy concrete about 30 m from the actual grave site. His statement needed verification. His shoes were analysed and the palynological assemblages on both of them showed that he had, indeed, picked up woodland palynomorphs, and the trees and shrubs were the same as those at the crime scene. But, only one of his feet yielded the assemblage characteristic of the gravesite itself; one foot had picked up components of Alnus (alder), Quercus (oak), Pinus (pine), Corylus (hazel), and other woody taxa and ferns. The other had the same assemblage and few grains of Hyacinthoides (bluebell). The floor of the woodland was carpeted with bluebells and Anemone (wind flower), and there were Rhododendron bushes next to the grave. If he had walked to the grave site, he would not have been able to avoid picking up larger amounts of bluebell pollen, that of Anemone and, possibly, Rhododendron on both feet. It would appear that he had picked up the woodland palynomorphs from the muddy concrete but there was no evidence that he had walked into the woodland with both feet. Only one yielded bluebell and this had probably been carried in soil to the concrete on the grave-diggers' feet. If both items of footwear had been amalgamated, the case for his non-involvement would have been weaker.

Evidence in a murder case in Greater Manchester was also enhanced by separate analysis of shoes (Wiltshire 2003d). Palynologically, this was a very complex case and resulted in my being cross-examined continuously for five days, and the accused being given a life sentence. The naked body of a woman, who had spurned her lover, was found lying on a path at a local woodland beauty spot. She had been beaten and there were foot marks on her face. One aspect of the case was to confirm a statement that events witnessed in the yard of the local public house might have been relevant. The victim had yellow stains on her jacket sleeve; the stains were composed of Forsythia pollen and green algae. The palynological assemblage from the suspect's shoes was shown to have a strong similarity to the actual crime scene a few hundred metres away, but one had a large number of grains of fenestrate Lactuceae (dandelion-like) pollen. The other shoe had only a couple of grains of dandelion-like pollen. A visit to the public house yard showed a concrete fence covered in green algae, with a flowering Forsythia bush growing over its top. There was a very narrow verge along the fence, dominated by dense growths of Taraxacum officinale (dandelion). Extensive searches and sampling of the local area failed to find anywhere which would offer such an assemblage of plants and palynomorphs in close proximity to one another. It was suggested that the suspect had started abusing the victim in the yard, pushed her against the fence, and whilst doing so, stepped on the verge and the dandelions with one foot. This was difficult for the accused to deny.

Mixed Samples: Digging Implements and Vehicles

Whenever buried remains are found, key exhibits will include digging implements. Unless a spade or shovel was bought for the criminal activity, it could have a palimpsest of soil layers distributed heterogeneously over the blade. Obtaining appropriate samples from such an item can be fraught with difficulty, and the best option might be to attempt a multiple sampling strategy. Again, a mixed sample will ensue and the skill of the palynologist can be severely tested in such cases. A soil-laden spade might also be laid on the ground where there is no bare soil but only a close cover of vegetation. Palynomorphs from the turf could dominate any number of profiles previously accumulated. Again, it is the rare or unusual assemblage of palynomorphs, or even a very rare palynomorph, that might indicate a link with a specific place. It is unlikely to be formed with widespread, common taxa.

In the case of the murder of Joanne Nelson, the 'Valentine Girl' (Wiltshire 2005a), her lover killed her but forgot where he had placed her body. His statement to the police was incoherent and they were anxious to find her remains. From palynological analysis of his vehicle, footwear, and a garden fork, I was able to eliminate his own, or his parents' garden, as being the source of the critical palynomorph assemblage. There were distinct similarities between the profiles from the car, one pair of shoes, and the garden fork and, from them, I was able to envisage the kind of place her body had been deposited and the vegetation of the place in question. The fork had not been used to bury her body but to cover it with woody, forest-floor litter. The signature of that litter was super-imposed on the pre-existing mud. Again, the soil on the exhibit was irrelevant to the investigation, and there was no soil from the crime scene on the fork. If soil analysis alone had been carried out, the girl would never have been located. Police found her body very quickly from the provision of an accurate description of the site. The assemblage of palynomorphs was an unusual one because of the nature of the Forestry Commission plantings; it also included spores of Polypodium (polypody) fern which is very uncommon in the area.

Seasonality and Temporal Interpretation

The time of an offence, or activities surrounding criminal activity, are often important aspects of police intelligence. Palynology has, on occasion, been used to confirm the temporal aspects of cases (Wiltshire, sub-judice cases ongoing).

The timing of anthesis (pollen release), especially of wind-pollinated plants, is critically important to those involved in studying allergy. Pollen and fungal spores are important allergens, and considerable effort is focused on pollen calendars. Such calendars are produced, and information exchanged, by various institutes, universities, and hospitals in many countries (Hyde 1969; Michel et al. 1976; O'Rourk 1990). The pollen calendars give start and finishing times, and the periods of peak release for pollen and spores for selected species. However, such calendars have limited use for forensic work because of the frequent, and sometimes extreme, variation in pollen release times from region to region.

In Britain there is a network of 10 pollen monitoring stations and 11 stations which monitor only grass pollen; the pollen stations are situated in towns in lowland areas, so their results may be unrepresentative of much of the British airspora. Even at a local level, 'pollen calendars' can never be precise – certainly, they cannot offer the precision required for forensic investigation. If the pollen calendar for a specific place were known in detail, there might be some application for interpretation of data relating to that place; but, because of specificity and inherent variability, the uses of seasonal records are very limited. They can only be used in the crudest way. For example, Montali et al. (2006) showed that pollen retrieved from corpses could not be related to the local pollen calendars because of the degree of variability in local conditions. However, they concluded that they could differentiate between winter/spring, spring/summer, and summer/autumn. To be sufficiently convincing to be useful in the forensic context, a great deal more work would be necessary and the phenomenon of residuality addressed. Depending on the environment, palynomorphs can remain in situ for very long periods. A soil sample might contain pollen accumulated over decades, and it can remain on foliage and bark for more than one year (Adam et al. 1967; Groeneman-van Waateringe 1998). Any pollen assemblage transferred during day-to-day activity would inevitably contain palynomorphs from a number of seasons. To rely on pollen calendar data for estimating seasonality in forensic work is imprudent.

Conclusions

In natural ecosystems, organisms occupy niches that may be narrow or wide. Some have a wide geographical distribution and others a narrow one. This is useful for predicting the nature of places from which palynomorphs were transferred to offenders. However, ecosystems are rarely natural in the true sense, with enormous environmental manipulation wherever people have had influence. From place to place, and sample to sample, palynological profiles are characterised by their variability and uniqueness. It follows that models for pollen dispersal and pattern of fall-out, which might aid the palaeoecologist in the interpretation of past environments where human intervention was minimal, will be of limited use in forensic case work. The amount of variation inherent in any system makes it impossible to construct databases of palynological profiles that could be used with confidence in criminal investigation and preparation of court statements. Every sample from every crime scene, and every assemblage retrieved from every exhibit, will be unique and will need independent evaluation. Predictions of origin of any organic particulate can only be crude, and there is no substitute for detailed analysis of the crime scene, other places pertinent to the investigation, and the objects that are thought to have had contact with them.

Worldwide, palynology is an under-used resource for criminal investigation. This is due, in part, to the perennial dearth of competent palynologists who possess not only comprehensive botanical knowledge, ecological training, and appropriate and extensive field experience, but who can cope with the rigours of cross-examination in the courts.

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