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Assessment of pollen assemblages on footwear for evidence of pollen deriving from a mock crime scene: a contribution to forensic palynology

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Abstract

To provide evidence of a link between a crime scene and a suspect, pollen analysis is occasionally employed. However, experimental research linking pollen on footwear to a specific crime scene has been infrequently undertaken such that there are limited references to cite in court. In this blind study, the author had to determine, which of 12 pairs of footwear had walked on a mock scene by comparing their assemblages to those of two scene controls. An additional four control pairs of footwear that had not been worn on or near the scene were also analysed. The pollen data from the footwear was assessed and compared with the scene control samples for: number of taxa, key types, Czekanowski Coefficient and general assemblages. Results indicated that six of the pairs of footwear had walked on the scene and these were, indeed, the ones that actually had done so. The four control pairs were dissimilar in most aspects, particularly key types. This research demonstrates, with some limitations, that evidence of a match between footwear sample and location can be found, even when material from other habitats is present on an exhibit.

Keywords: *pollen spectrum, footwear exhibits, palynomorphs*

Analysis of pollen on footwear is occasionally used in some serious crime cases in order to provide a link from a suspect to a particular scene, typically a body deposition site. Pollen, fungal spores and other biological entities, together termed palynomorphs, can occur in soil samples in high amounts and with differing assemblages, depending on the location. Since palynomorphs can endure in the environment for a long time, are microscopic and morphologically and spatially variable, they can provide useful trace evidence (Miller Coyle 2005; Walsh & Horrocks 2008; Sandiford 2012), although the skills and techniques required for forensic palynology are complex and require experience (Adams-Groom 2012).

In crime cases using palynology, footwear will be analysed when it is suspected that it has been worn on a scene and potentially collected palynomorphs, either by direct contact with pollen-bearing plants or by transference of soil. There are many factors that influence the dispersal of pollen from a plant and its

subsequent arrival and duration in the soil, such as climate, time of year, soil type, land-use, climate, habitat type and chemical, bacterial, fungal and faunal action (Spicer 1991; Davidson et al. 1999; Van Mourik 2003). The quantity of pollen or spores produced by the local plants and fungi, along with their dispersal strategies, also influence the assemblage (Traverse 1994; Mathias et al. 2012).

There can be great heterogeneity in samples taken from crime scenes, although in the United Kingdom (UK), there are several plants (*Alnus*, *Betula*, *Pinus*, *Quercus* and *Poaceae*) whose pollen appear in almost every sample because they are commonly occurring, wind-pollinated and produce pollen in very high concentrations (Adams-Groom 2015). The presence of these types on an item is therefore of lower value compared to that of less frequent types. There are also a number of other palynomorphs that often contribute to assemblages but usually in low amounts. Where these appear in un-

sually higher amounts, their incidence may be of more value. Then there are the rarer types which are specific to a scene because they are not dispersed by the wind and are produced in low amounts. Such types may only occur in small concentrations in a sample but may be of greater value in the assemblage (Nguyen & Weber 2015).

Wiltshire (2006) noted that an assemblage of palynomorphs obtained from an item of footwear will never match any scene perfectly since it could have accumulated them from any number of habitats prior to sampling from it. Transfer of palynomorphs from scene to footwear may also be affected by the site conditions. Wet soil is sticky and will adhere more readily to surfaces while dry, dusty soil will transfer in lower concentrations. Other factors such as length of time between crime and seizure of footwear, sole form and shoe cleaning by suspect will all affect the chances of a good match (Adams-Groom 2012). A discussion on the advantages and limitations of analysing footwear for trace evidence can be found in Morgan et al. (2009).

Only a few authors have discussed the presence and variability of pollen in soil relating to forensic palynology. Horrocks et al. (1998) compared pollen from samples taken from an open grassy area 15 m × 6 m with shoeprints obtained from the same area and found a high degree of similarity between all of them, suggesting homogeneity for that particular habitat type.

Experimental research comparing footwear pollen samples to a specific crime scene has been quite limited. Horrocks et al. (1999) compared pollen spectrums on shoeprints with controls collected at the same location and found close similarity. Nguyen and Weber (2016) undertook experimental research on the collection of pollen from an indoor environment. The shoes in both of these pieces of research were clean, however, while in most real cases, multiple exposure would have occurred. Some forensic palynologists have presented case studies involving footwear in peer-reviewed articles, e.g. Mildenhall (1990), Horrocks and Walsh (2001), Wiltshire (2006) and Wiltshire et al. (2014), 2015). Other palynological research related to footwear includes a study by Riding et al. (2007) who examined the pattern of how footwear worn at a number of sites collected and retained soil pollen assemblages. They found that where mixing of soil from different sites occurred on the footwear, it was the spectrum of the last site that tended to be the most dominant. Of course, in real crime cases, the palynologist is unlikely to know where the footwear has been worn apart from the potential for it to have been worn at the crime scene(s). Bull et al. (2006) analysed material from the cast of a footprint in a

murder case and were able to determine from pollen, fibre, chemical and physical soil components that the wearer had been standing recently in a nearby stream and could reconstruct three phases of previous activity for the footwear item before it attended the crime scene.

This study analysed a range of footwear that had trodden on any number of unknown locations in the past and some of which were subsequently worn at a mock crime scene. The aim was to determine, which items of footwear had trodden on the scene by comparing their soil pollen assemblages with control samples from the same location and to provide an empirically tested methodology.

Material and methods

Sampling the 'scene'

All sampling was undertaken on 14 February 2011. Rain had fallen the previous day and the soil was damp. A mock scene, where a clandestine grave could have been, was selected on the campus of the University of Worcester, in a relatively quiet area only accessed occasionally by staff and students. This location comprised a good variety of plants with varying pollen dispersal strategies. The scene was dominated by two large plane trees and pollen from this type, although wind-pollinated, tends to be found only in very low amounts in samples except where the source tree is in fairly close proximity. The chosen scene was a small 'L-shaped' conservation area, 52 m × 48 m in size. It was contained on two sides (east and south) by residential fencing with small suburban gardens beyond, to the north-west lay an extensive teaching block rising to three storeys with a path around it and lawn and hedging lay to the west. In the northern section of the scene itself, there were dense immature trees and a pond and in the western section lay an open meadow. The plane (*Platanus × acerifolia* [Aiton] Willd.) trees lay in the southern section and a mature Italian Cypress tree (*Cupressus sempervirens* L.) near the centre. Other vegetation observed at the scene and within 20 m of it, in February, included: *Acer campestre* L., *Acer pseudoplatanus* L., Apiaceae, *Arum maculatum* L., *Betula pendula* Roth, *Cedrus* sp., *Clematis* sp., *Corylus avellana* L., *Crataegus monogyna* Jacq., *Crocus* sp., Cupressaceae, *Fraxinus excelsior* L., *Hedera helix* L., *Hyacinthoides non-scripta* (L.) Chouard, *Jasminum nudiflorum* Lindl., *Kerria japonica* (L.) DC., *Lonicera* sp., *Morus nigra* L., Poaceae, *Prunus laurocerasus* L., *Prunus* sp., *Ranunculus* sp., *Salix* sp., *Sambucus nigra* L., *Sorbus torminalis* (L.) Crantz, *Taxus baccata* L., *Urtica dioica* L.

Table I. Summary of the descriptions of material visible on the footwear during examination with information about tread pattern and which items had been exposed to the scene.

Sample	Footwear description	Material description	Exhibit	Control	Walked on scene
ABC/1	Boots with tread pattern on sole.	No significant visible material.	✓		
ABC/2	Walking boots with visible signs of wear and right boot retaining very little tread	Fair amount of dark brown soil and some pieces of vegetation	✓		✓
ABC/3	Boots with 3 cm heel and fine ridge pattern on heel sides.	Small amounts of brown soil noted on sole.	✓		
ABC/4	Trainers with tread pattern on sole	Small rocks in tread and small particle of debris	✓		
ABC/5	Boot with tread pattern on sole	Small amounts of debris on both boots, small amount of light brown soil on left boot	✓		
ABC/6	Ankle boots with fine grooves on sole.	Small amounts of brown soil and small vegetation pieces.	✓		
ABC/7	High top pumps with dotted pattern on sole.	Several large deposits of brown soil on soles.	✓		✓
ABC/8	Trainers with ETNIES logo on sole.	Nothing noted by examiner but sample collected.	✓		✓
ABC/9	Walking boots, quite worn but with tread pattern present	Large deposits of dark brown soil, large sand particles & pieces of vegetation between the treads	✓		✓
ABC/10	Leather boots with tread pattern on sole	Mid brown soil found between treads plus vegetation pieces	✓		
ABC/11	Walking boots with thick tread pattern	Large quantity of brown soil on both boots plus pieces of vegetation	✓		✓
ABC/12	Mule shoes, well worn, little tread pattern	Brown sandy soil on tread with very small amounts of vegetation pieces	✓		✓
NS/1	Walking boots with thick tread pattern	Large amounts of sandy loam soil and pieces of vegetation		✓	
NS/2	Trainers with tread pattern on sole	Stains of reddish soil & small deposits of a darker soil with fragments of grass		✓	
NS/3	Trainer shoe, well worn, some tread pattern retained	Dark brown clay type soil and grassy deposits present		✓	
NS/4	Trainers with tread pattern on sole	Reddish brown soil and greyish brown soil deposits and pieces of grass.		✓	

Two control samples were taken from the central area of the scene for comparison to the samples from the footwear: Control A was taken from the main access point near the path and Control B at 7 m further south-west. The samples were taken by scraping approximately 4 cm³ of the sandy loam soil to a maximum depth of 5 mm from an area covering 1 m² for each control.

175 *Sampling from footwear*

175 On the same day, 12 student participants visited the scene wearing footwear brought in especially for the project. They had been asked to choose any type of footwear, clean or dirty, so that the experiment would be as close to reality as possible. All the students regularly attend the University but the author did not know whether or not any of these particular items of footwear had previously been worn on the campus or indeed at the scene itself, since this is not information that a forensic palynologist would

usually have about a suspect's footwear. The students changed into the footwear in a classroom and then walked to the scene 119 m distant. It was requested that some of the students walked across the central area of the scene and trod on some or all of the control sampling areas and that the others remained off the scene. The students decided amongst themselves who would walk on the scene. All students then walked back to the classroom, removed their footwear and then examined it for deposits. Each student produced an examination document describing the item, its condition and the presence and type of any material on it, which is summarised in Table I. The students then removed the deposits to test tubes by scraping and washing with a warm 3% detergent solution and toothbrushes. To reduce bias in the analysis, a technician was asked to secretly allocate a sample number to each student's sample and to note, which of them had walked on the scene. This information was not made available to the author until her comparative analysis was complete. However, the

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author knew that at least some of the footwear was likely to have walked on the scene so there was always some element of bias. The author also supplied a sample from a pair of shoes regularly worn at the University and which may have walked at the scene at some point in the past, and the technician allocated sample number ABC/6 to these. Footwear items that could have walked on the scene are referred to in the results as ‘exhibits’.

Sampling from non-scene control footwear

Four items of footwear that had never been worn at the University of Worcester were supplied by visiting guests, on request, for analysis as random control samples (NS/1–4, Table I). These items of footwear had previously been worn at any number of unknown locations. The items were scraped and washed in the same manner as the previous set.

Processing of samples and pollen identification

All samples were processed using standard palynological techniques of digestion, acetolysis and heavy liquid separation to separate the pollen from the soil matrix (Moore et al. 1991; Brown et al. 2008). The resulting pollen pellets were mixed with glycerine gelatine mountant and the mixture applied to microscope slides. Using brightfield microscopy, the slides were then sampled for different pollen types by counting longitudinal transects randomly across each slide until a minimum of 300 grains per sample had been obtained. This is the minimum number considered to be viable for reliable results in palynological analysis of soil samples. However, sample NS/1 contained very little pollen and only 235 grains were obtained. Pollen and fern spores were counted but not fungal spores, largely for reasons of simplicity in this study since they can be very numerous in type. The results were tallied and converted into percentages for statistical analysis.

Comparative analysis

The pollen assemblages resulting from the analysis of the footwear samples were compared to those of the controls in several ways to draw out the similarities and differences between them. Since coincidental similarities are possible between pollen assemblages, it is important when analysing data that may be used as admissible evidence to look at as many aspects as possible. These aspects, in this study, were as follows:

1. The number of taxa found in each separate scene control and the two scene controls com-

bined and averaged, were compared with those found in each sample from the footwear.

2. For the control samples, the ‘key’ types were identified. These are pollen taxa that are either site-specific, rare or were encountered in unusually high amounts and which should be present in reasonably similar amounts in the samples if they originated from the scene.
3. A visual assessment of the overall assemblage of each sample compared to the controls was undertaken and a summary composed. The author looked for general similarity to the controls throughout the sample, both in types present and amounts and it was necessary to determine whether or not material from other locations was present because this could interfere with the strength of the decision.
4. Statistical comparative analysis can help to further inform or confirm the extent to which the samples are in common with the controls. In this case, the Czekanowski Coefficient was chosen because it determines similarities between taxa in common. The resulting Similarity Index (SI) is an ordination from 0 to 1 where 0 indicates no similarity and 1 indicates the two samples are identical. A result greater than 0.5 would suggest some similarity, 0.6–0.75 moderate similarity while a result above 0.75 would suggest a high similarity.
5. A final assessment table was prepared summarising the findings of all of the earlier mentioned. A decision was made as to whether or not each sample could have derived from the mock scene and the strength of the decision was also considered.

Common airborne pollen types

Pollen data from the ambient atmosphere is collected from a number of pollen monitoring stations around the UK as previously documented in, for example, Emberlin (1997) and Corden et al. (2000). These stations monitor pollen types that trigger allergy and many also record non-allergenic types which frequently occur. At the University of Worcester, there is a permanent pollen monitoring trap (part of the UK’s pollen monitoring network), which is located approximately 150 m from the mock scene. Since it is helpful to know, which pollen types are prevalent in the local airstream at a crime scene where palynology is to be used as evidence, the yearly catch for relevant taxa has been presented. It should be noted that not all pollen types found in forensic soil samples are encountered in air samples due to differing pollination strategies.

Table II. Pollen count results for the control samples A and B, the mean of the control samples combined and the exhibits.

Taxon	Control		Controls		ABC/		ABC/		ABC/		ABC/		ABC/		ABC/		ABC/		ABC/		
	A	B	A + B	A + B	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
Deciduous trees																					
<i>Acer</i>	1	1	1	1	2	3	3	3	1	1	13	1	13								
<i>Aesculus</i>	1	1	1	1	15	1	1	1	1	1	1	1	1								
<i>Alnus</i>	3	3	3	3	4	11	30	37	36	13	21	10	17	10	5	6	6	7	7	1	
<i>Betula</i>	1	2	2	2	106	6	13	4	10	12	10	7	10	109	4	7	4	17	1	13	
<i>Carpinus</i>					2						1	1									
<i>Carya</i>					1								1								
<i>Gastanea</i>					1					1											
<i>Corylus</i>	2	4	3	3	1	18	20	34	53	47	49	15	17	5	3	6	3	18	2	3	
<i>Fagus</i>		5	3	3	2	2	64	3	1	1	1	1	2	1	1	1					
<i>Fraxinus</i>	4		2	2	51	2	2	2	6	12	2	4	4	5	2	1	2			14	
<i>Juglans nigra</i>										1											
<i>Morus nigra</i>	6		3	3			30	8	1	1	10	1				19					
<i>Platanus</i>	25	123	74	74	2	90	7	5	5	1	52	83	99	3	75	39					
<i>Populus</i>					3							1									
<i>Quercus</i>	1	4	3	3	6	2	3	12	7	8	4	3	7	1		2	2	16	37	8	
<i>Rosaceae</i>	2		1	1	5	4	1	3	3	3	3	2	43	1	2	2	2	2	2	2	
<i>Salix</i>	1	1	1	1	2		1		1	1			2	1	1	1				5	
<i>Tilia</i>	3	1	2	2	3	2	1	4	1	16	9	9	1		2						
<i>Ulmus</i>	1	1	1	1	1	1	4	5	13	1	702	613		1	6						
Coniferous trees																					
<i>Cedrus</i>	6	4	5	5	2	1	2	1	2	8	1	1	2		4	3					
<i>Cyprioneria japonica</i>							5		2												
<i>Cupressaceae</i>	70	8	39	39	29	3	10	12	19	4	7	6	10	2	7	5					
<i>Larix</i>	3		2	2	10			11	3		4	1			11	1				1	
<i>Picea</i>										1				1							
<i>Pinus</i>	19	16	18	18	14	26	12	12	10	7	21	16	22	5	37	39	20	29	8	15	
<i>Sequoia t.</i>										1	1										
<i>Taxus baccata</i>	2	1	2	2	6		43	108	116	4	2	3	28	2	4	18					
Shrubs, climbers, evergreens																					
<i>Ericaceae</i>																					
<i>Eucalyptus</i>	1	2	1	1	2	2		1	1	2	1				1	1				2	
<i>Hedera helix</i>													1		6	6					
<i>Ilex aquifolium</i>	1	1	1	1	13	1	6	4	1	1	4	1	1							1	
<i>Ligustrum vulgare</i>	1	1	1	1	1	1	1	1	2	2	1									1	
<i>Lonicera</i>										1											
<i>Rhamnus</i>										5	3	4									
<i>Sambucus</i>					4					2			27								

(Continued)

Table II. (Continued).

Taxon	Control		Both controls	ABC/												NS/			
	A	B		1	2	3	4	5	6	7	8	9	10	11	12		1	2	3
Apiaceae	2	6	4	1	6	1	1	1	1	1	1	1	1	1	1	2	2	9	2
<i>Artemisia</i>				1													2		
Asteraceae: liguliferous type	79	19	49	5	10	6	5	2	11	11	11	8	5	33	45			2	23
Asteraceae: tubuliferous type	1	2	2	1	1	2	4	4	11	1	1	1	2	2	2	8	2	5	26
Brassicaceae	3	2	3	1	1		2	2	3			3	5	7	4			2	
Caryophyllaceae									1				1					2	
<i>Chenopodium</i>	1	2	2	2	1	1	11	2	1	1	1	1	2	2	2			2	1
Cyperaceae																			
Euphorbiaceae																			
Fabaceae				1			1					1				1			
<i>Impatiens sp.</i>				1	2	2				1	2		2		3				
<i>Impatiens glandulifera</i>				2			15	2		4		1							
Lamiaceae				1			1	2											
<i>Lamium</i>								2											
Liliaceae	1	1	1							1									
Malvaceae				1															
<i>Mercurialis annua</i>				1															
<i>Myosotis discolor</i>												6							6
<i>Plantago</i>	1		1	1			1		1				1				1		25
<i>Plantago lanceolata</i>		1	1	1								1							1
<i>Plantago major</i>				1			2						3						185
Poaceae	42	105	74	25	104	45	82	21	140	41	75	46	221	102	79	151	252	215	185
<i>Polygonum</i>							1		2	1	1	1	2			22	1		2
Ranunculaceae							1		2	1	1	1	3						2
<i>Rumex</i>				1			1		1	1	1	1	1			3	3		1
<i>Trifolium</i>												1	1			1			
<i>Typha</i> t.																			
Urticaceae	10	4	7	2	5	5	1	3	11	1	4		7	7	21	3			2
Ferns and mosses																			
<i>Asplenium</i>									1										
<i>Polypodium</i>																		1	
<i>Pteridium aquilinum</i>	13		7						28	5									78
<i>Sphagnum</i>		1	1						1										
Total for sample	306	321	314	315	314	333	392	338	339	999	886	378	388	315	333	235	348	288	425

Note: Key types are highlighted.



Also, pollen can be transported to a scene via people on footwear, clothing and in some cases, vehicles. This mock scene is in a quiet area of the campus, is walked over intermittently and mown once a year.

Results

A wide variety of footwear types were worn in the study and all but three (ABC/1, 4, 8) were described as containing at least some soil and vegetation pieces while exhibits ABC/2, 7, 9, 11 were described as having large deposits of brown soil (Table I).

Pollen types considered to be of particular comparative importance in the control assemblages, i.e. the 'key' types, were: *Morus nigra*, *Platanus* sp., Cupressaceae and Asteraceae: liguliferous type (Table II). Controls A and B were found to be dissimilar in the concentrations of these key types and their overall pollen assemblages were also different to a certain extent (Table II), despite being only a few metres apart. Comparing the controls to the exhibit samples visually (Table II), *Platanus*, which is present in all the exhibit samples, occurred in amounts comparable to the controls in only six of the samples: ABC/2, 7, 8, 9, 11, 12 and was not found in the non-scene samples (NS/1–4). *Morus nigra* was found in ABC/3, 5–8, 12. Cupressaceae occurred in all the exhibit samples with some comparable to Control B: ABC/3, 7–9, 11, 12, but not to Control A. Asteraceae: liguliferous type occurred in all exhibit samples but compared to the controls, only ABC/11, 12 had similar amounts to the com-

bined controls, while ABC/2, 6–8 bore moderate similarity to Control B.

Assessing all factors together, including the results from the coefficient analysis for the taxa in common, samples from exhibits ABC/2, 7–9, 11, 12 were considered to have derived from the scene (Tables II, III, V). These samples did indeed prove to be those that had actually walked on the scene (Table I). ABC/2, 11, 12 bore the strongest similarity to the scene. ABC/7, 8 only had an overall moderate similarity to the scene because the similarity indices were low. All the other exhibits were more dissimilar than similar in most, if not all of the four aspects.

Sample ABC/6, the author's own footwear, did not produce a good match (Tables II, III, V). All the key types were present, as might be expected, but they were in different amounts, particularly *Platanus* where only one grain was found. In addition, the general assemblage amounts were different and the coefficient analysis showed no similarity.

The pollen data from the air samples taken at the University of Worcester (Table IV) clearly shows that Urticaceae, Poaceae, *Quercus*, *Betula*, *Fraxinus* and *Alnus* are prevalent and that their pollen would be present in most soil samples taken in the area. As a result, these pollen types carry less weight in the assemblages than others. All the types in Table IV are wind-pollinated apart from *Salix* and *Tilia*, which are primarily insect-pollinated but can also be wind-dispersed (termed 'amphiphilous').

Table III. Similarity indices from Czekanowski Coefficient analysis on controls, exhibits and non-scene controls.

	Control A	Control B	Controls A + B
Control A	—	0.47	—
Control B	0.47	—	—
ABC/1	0.38	0.30	0.30
ABC/2	0.44	0.84	0.70
ABC/3	0.39	0.38	0.34
ABC/4	0.34	0.41	0.40
ABC/5	0.30	0.24	0.28
ABC/6	0.38	0.50	0.45
ABC/7	0.21	0.19	0.21
ABC/8	0.28	0.28	0.28
ABC/9	0.44	0.64	0.58
ABC/10	0.26	0.44	0.36
ABC/11	0.55	0.82	0.79
ABC/12	0.60	0.62	0.68
NS/1	0.44	0.66	0.60
NS/2	0.38	0.66	0.54
NS/3	0.28	0.56	0.44
NS/4	0.44	0.75	0.54

Note: Bold type indicates more similarity than dissimilarity for the taxa in common.

Discussion

Control A was located nearer to the *Morus nigra* tree than Control B and very close to the *Cupressus sempervirens* tree, whereas Control B was further away from both of these but directly beneath the canopy of one of the *Platanus* trees. Even though the control samples were only 7 m from each other, there were obvious differences in the assemblage of each depending on proximity of the representative plants.

Ulmus pollen was present in extremely high amounts in samples ABC/7, 8 and over-counting was required to obtain comparable amounts of the other types. Since there was not an elm tree in the vicinity of the scene, it is clear that this material was not collected from it. Indeed, evidence of multiple assemblages can be seen on many of the exhibits, either as additional pollen types or in concentrations far exceeding those found at the scene. Very high amounts of *Ulmus* and several other tree types, which were only present in low amounts in the controls, skewed the statistical analysis producing only low SIs for these two samples. Sample ABC/9 was

deemed to be generally similar in most aspects to the scene but with only a limited strength rating because, as with ABC/7, 8, it is clear that material from other locations was also present in the sample and this has tended to obscure the scene assemblage and increase the chances of a false positive.

Although the exhibit footwear had been worn in and around the Worcester area and most of them on the University Campus as well, it was nevertheless possible to distinguish those that contained the mock scene profile. Various footwear types had trodden on the scene (ABC/2, 9, 11 were walking boots, ABC/7 were pumps, ABC/8 were trainers and ABC/12 were mule shoes), but all had collected sufficient sample for successful analysis. However, there are a number of limitations inherent in this study, as follows: Firstly, the soil on the scene was damp and this would have increased the chances of pick-up on the footwear and ultimately improved the likelihood of a match being found. Secondly, when the footwear was cleaned to obtain sample, different soil types were not isolated, whereas in a real case, they might be. It is not always possible to distinguish different soils by eye, but where possible, this is done and it helps reduce interferences from other assemblages on the exhibit. It should also be noted that even footwear that has no visible soil deposits may yield some palynomorphs. Thirdly, the time-lag between the footwear leaving the scene and obtaining samples from them was minimal compared to what it might be in reality, as was the distance between scene and laboratory. Finally, the students were asked to walk across the section of ground from where the controls were taken, thus increasing the chances of a good match. This is the logical approach for the type of crime that this study was simulating, i.e. a clandestine grave where the murderer must have trodden. However, in a real crime scenario, it may be necessary to take many more control samples due to the heterogeneity of a scene area (Horrocks et al. 1998), or where it may be unclear where a suspect could have walked.

Since pollen and the plants that produce it are subject to so many variables, pollen analysis of this type can be very complex. The four aspects used in the analysis here each helped to contribute elements of information to enable the decision-making process but there are inherent problems with each of these, as follows.

Number of taxa in common with controls

The number of taxa that each sample has in common with the controls could be expected to be

Table IV. Pollen types recorded at the University of Worcester showing the mean yearly catch for the five years up to and including 2011.

Taxon	Mean catch	Pollination period
Urticaceae (Nettle family)	6878	June–September
Poaceae (Grass family)	5199	May–August
<i>Quercus</i> spp. (Oak)	3432	April–June
<i>Betula</i> spp. (Birch)	3300	March–May
<i>Fraxinus</i> spp. (Ash)	1780	March–April
<i>Alnus</i> spp. (Alder)	1506	February–March
<i>Corylus</i> spp. (Hazel)	547	February–March
<i>Salix</i> spp. (Willow)	471	March–April
<i>Platanus</i> spp. (Plane)	437	March–April
<i>Plantago</i> spp. (Plantain)	153	May–August
<i>Rumex</i> spp. (Dock)	152	June–July
<i>Castanea</i> sp. (Sweet chestnut)	151	July
Amaranthaceae (Pigweed family)	95	August–September
<i>Ulmus</i> spp. (Elm)	78	March–April
<i>Artemisia</i> (Mugwort)	59	July–August
<i>Tilia</i> spp. (Lime)	49	June–July

greater in samples containing material that originated from the scene compared to those that did not. However, a sample deriving from footwear that has obtained material from several locations is also likely to have a fairly high number of taxa in common too. This is because many types are so common in the environment generally (Adams-Groom 2015). Also, since many taxa occur in trace amounts, it is unlikely that the number in common would exactly match since some may not have been picked up. Nevertheless, this aspect can be useful in the decision-making process since a sample with around half or less than the number of taxa in common with any of the controls is unlikely to have derived from the location of interest.

Visual assessment of the overall assemblage compared to controls

The season in which the samples were collected was late Winter when *Alnus*, *Corylus*, *Ulmus* and *Taxus* were approaching their peak emission periods (Emberlin et al. 2007; Skjøth et al. 2015). Footwear worn regularly in this season could readily collect airborne pollen types. That is why these particular taxa were frequently higher in the exhibit samples than those from the scene, where these tree types were not flowering in the immediate vicinity.

Key types

Four key types were identified from the controls in this case, either because they are uncommon or because they occurred in unusually high amounts.

475 The key types cannot stand alone as evidence types
 unless they are very rare and must be considered
 both as a suite of key types and as a part of the
 more general assemblage in question. Common
 types carry a lower value than rarer types and their
 480 concentrations within any assemblage must be
 assessed and carefully compared to the comparator
 samples (Adams-Groom 2012). The four key types
 identified as important to this case were chosen due
 to the following reasons.

485 *Platanus*. – The amount of pollen produced by
 plants varies temporally and spatially due to various
 factors but estimates have been made for some trees
 (Molina et al. 1996; Broström et al. 2008), includ-
 ing *Platanus*. Molina et al. (1996) counted and cal-
 490 culated the numbers of flowers and pollen grains on
 a number of wind-pollinated trees. For *Platanus*, for
 one year and three trees, they calculated a range of
 188.4×10^8 to 302×10^8 pollen grains per metre of
 crown. Even with these large productions, most pol-
 495 len is likely to fall within several hundred metres
 from the source (McCartney 1994; Skjøth et al.
 2013; Sofiev & Bergmann 2013), mainly because
 pollen grains are heavier than air and will drop to
 the ground quickly in still air or only light airflow
 500 (Gregory 1961). Bricchi et al. (2000) found that
 about a quarter of all the pollen emitted from a
 lone plantation of *Platanus* trees in Italy fell in an
 area within 400 m of the source and the great major-
 ity within 800 m.

505 Adams-Groom (2015) examined the frequency
 and abundance of pollen types in 199 UK crime
 case samples and found that *Platanus* pollen
 occurred in 23.3% of them and at a mean concen-
 tration of 1.28%. Therefore, once larger amounts
 510 are found, it is possible that the source tree is in
 the vicinity while high amounts suggest the source is
 very close. In the case of most wind-pollinated trees,
 this information may be of only limited value
 because they are so common but since *Platanus* is a
 515 non-native species in the UK with restricted distri-
 bution, the presence of its pollen in high amounts
 carries greater weight.

Morus nigra. – This is an ornamental tree found
 occasionally in parkland and gardens so its pollen is
 520 unlikely to be found in a soil sample except in close
 proximity to where the plant is growing. Adams-
 Groom (2015) found this type in only 1.7% of sam-
 ples at a mean abundance of 0.34%. There is only
 one *Morus nigra* tree on the University of Worcester
 525 campus and it overhangs the path that the students
 walked along to reach the scene and there are cracks
 in the path where soil accumulates. Two samples
 from footwear (ABC/3, 5) did not tread on the
 scene had *Morus nigra* pollen. It is possible that
 530 this footwear picked up the *Morus* pollen from walk-

ing along the path or collected it in secondary trans-
 fer from soil that had fallen off footwear of students
 that had already walked along the path after treading
 on the scene.

Cupressaceae. – The Cupressaceae family is large 535
 with many non-native, widely-planted ornamentals
 whose pollen is found in about 50% of samples,
 usually in low amounts (average 1.37%; Adams-
 Groom 2015). Pollen from the different genera
 540 within this family is morphologically similar and,
 because in soil samples they are often found in
 poor condition, it can rarely be assigned to genus.
 It has been included as a key type because the family
 member *Cupressus sempervirens* tree grows very close
 to the site from which the controls were taken and
 545 unusually high amounts of Cupressaceae pollen
 were found in the controls.

Asteraceae: liguliferous type. – This is the dandel-
 550 lion-type pollen, which is often found in soil samples
 (70.7%) but in low percentages at an average of
 2.35% (Adams-Groom 2015). It occurred in the
 controls in uncommonly high amounts and there-
 fore became a key type where found in association
 with other key types. Because this type is frequently
 555 found, however, its value in a sample should be
 treated with caution and only considered where it
 is clear that it has a contribution to make.

Statistical analysis

560 Various forms of statistical analysis could be used to
 help inform the decision-making process. However,
 statistics will only expose numerical variances and
 cannot highlight important environmental elements
 such as the value of the presence of certain taxa
 within the profile. For example, amounts of grass
 565 (Poaceae) pollen may be very similar and increase
 match probability, as happened for the control exhib-
 its (NS/1–4), but this type has low value because it
 is so common and is found in every sample. More-
 over, many statistics will only compare similarities
 570 between taxa that are in common between samples
 but the entire profile must also be assessed. The
 main point is that the use of statistics may not be
 probative in the pollen context and that the most
 emphasis should be placed on the key types as well
 as the overall profile. In this case, some of the sam-
 575 ples contained high amounts of some tree taxa,
 which skewed the results of the coefficient analysis,
 notably high amounts of *Ulmus* and several other
 tree pollen types in ABC/7, 8. The key types indi-
 580 cated that the samples were likely to have derived
 from the scene but the coefficient analysis did not
 concur because of the high amounts of these few
 taxa that had been picked up from another location.

Table V. Summary of assessment of data and statistical analysis to determine whether or not footwear pollen samples originate from the mock scene.

Exhibit	Number of taxa in common with controls		Key types found and a qualitative assessment of similarity in <i>Morus nigra Platanus sp.</i> Cupressaceae Aster: lig. t.			Visual assessment of overall profile of sample compared to controls	Assessment of coefficient results	Decision	Strength of decision
	A	B	Not present	Low	Moderately similar to A				
ABC/1	26/30	22/27 29/37	Not present	Low	Moderately similar to A	Too low	Dissimilar	No	Moderately Strong
ABC/2	22/30	19/27 24/37	Not present	Comparable with B	Comparable with B	Moderately similar to B	Dissimilar to A, similar to B	Yes	Moderately Strong
ABC/3	20/30	17/27 21/37	Yes, but much higher	Low	Comparable with B	Low	Dissimilar	No	Moderate
ABC/4	22/30	21/27 26/37	Not present	Low	Comparable with B	Low	Dissimilar	No	Moderate
ABC/5	23/30	20/27 26/37	Comparable with A	Low	Moderately similar to A	Low	Dissimilar	No	Moderate
ABC/6	24/30	16/27 23/37	Low	Low	low	Moderately similar to B	Dissimilar	No	Moderately Strong
ABC/7	25/30	20/27 27/37	Comparable with A	Comparable with both	Comparable with B	Moderately similar to B	Invalid: high <i>Ulmus</i> skewed results	Yes	Moderate
ABC/8	21/30	19/27 23/37	Low	Comparable with B	Comparable with B	Moderately similar to B	Invalid: high amounts of <i>Ulmus</i> skewed results	Yes	Moderate
ABC/9	18/30	20/27 21/37	Not present	Comparable with B	Comparable with B	Low	Low/Moderate similarity	Yes	Limited
ABC/10	14/30	13/27 15/37	Not present	Low	Low	Low	Dissimilar	No	Strong
ABC/11	19/30	19/27 21/37	None	Comparable with both	Comparable with B	Comparable with both	High similarity to B	Yes	Strong
ABC/12	22/30	19/27 24/37	Yes, but higher	Comparable with A	Comparable with B	Comparable with both	Low to Moderate similarity	Yes	Moderately strong



NS/1	10/30	10/27	12/37	None	None	None	None	Low number of types in common, no key types. High Poaceae gives low-value similarity to B.	Low similarity to B	No	N/A
NS/2	10/30	7/27	10/37	None	None	None	None	Low number of types in common, no key types. High Poaceae gives low-value similarity to B.	Low similarity to B	No	N/A
NS/3	11/30	10/27	11/37	None	None	None	Low	Low number of types in common, key types dissimilar. High Poaceae gives low-value similarity to B.	Low similarity to B	No	N/A
NS/4	17/30	13/27	19/37	None	None	None	Comparable with B	Low-value similarity for Poaceae and Asteraceae compared to Control B, otherwise no similarity.	Moderate similarity to B	No	N/A

Note: ~~Controls A and B combined are denoted with '&'~~ Scale of support: Neutral; Limited; Moderate; Moderately Strong; Strong; Very strong; Extremely Strong (Jackson & Jackson 2004).

In a real case, the palynologist would also consider whether or not the suspect could have collected similar material to the crime scene from an alternative location and would need to know where the suspect lived and worked or took outdoor exercise. Other scenes may be analysed and compared to the exhibits and evaluated accordingly (Adams-Groom 2012).

Conclusion

For locations that have a diverse pollen assemblage, this research demonstrates that evidence of a match between footwear and a location can be found when the exhibits are seized quickly and even when they contain material from other habitats. The palynological evidence would be even stronger if used in conjunction with other environmental assessments conducted at the same time, such as soil analysis. However, the strength of the evidence was only strong for one pair of shoes. For the others, the evidence was only perceived as moderately strong or moderate and for one pair, limited. This highlights the complexity of pollen analysis due to lots of variables and reinforces the need for the palynologist to consider multiple aspects before returning admissible evidence. Further research would look at expanding the time period between access to the scene and seizing of the footwear and focus on tread variation.

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