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Differential retention of pollen grains on clothing and the effectiveness of laboratory retrieval methods in forensic settings

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Highlights

- Pollen retention on clothing after a period of light or heavy wear is tested.
- Retention patterns are complex. Species and fabric characteristics are important.
- Standard washing procedures for removing pollen from fabric are inconsistent.
- These are crucial findings for all forensic investigations using palynology.

Abstract

Forensic palynology has been important in criminal investigation since the 1950s and often provides evidence that is vital in identifying suspects and securing convictions. However, for such evidence to be used appropriately, it is necessary to understand the factors affecting taphonomic variability (i.e. the variability in the fate of pollen grains before they are found during forensic examination). Here, we test the relative amount of pollen retained on clothing after a period of simulated light or heavy wear based on pollen and fabric characteristics. We also test the efficiency of forensic laboratory protocols for retrieving pollen from fabrics for analysis. There was no statistically significant difference in retention of fresh or dried pollen on any fabric type. There was a substantial difference in pollen retention according to wear intensity, with considerably more pollen being retained after light wear than after heavy wear. Pollen from insect-pollinated species was retained at higher concentrations than pollen from wind-pollinated species. This pattern was consistent regardless of wear intensity but pollination type explained more of the variability in pollen retention after light wear. Fabric type was significantly related to pollen retention, but interacted strongly with plant species such that patterns were both complex and highly species-specific. The efficiency of removing pollen with the standard washing protocol differed substantially according to plant species, fabric type, and the interaction between these factors. The average efficiency was 67.7% but this ranged from 21% to 93%, demonstrating that previous assumptions on the reliability of the technique providing a representative sample for forensic use should be reviewed. This paper highlights the importance of understanding pollen and fabric characteristics when creating a pollen profile in criminal investigations and to ensure that evidence used in testimony is accurate and robust.

Keywords

Palynology, pollen analysis, forensics, laboratory protocols, criminal investigation, expert witness, fabric, clothing, garments.

Introduction

Pollen produced by sexually reproducing plants is both ubiquitous and resilient in the environment. As well as being associated with parent plants, pollen grains from the species growing in a particular area are found in the air, soils and sediments within that locale. Thus, although there is always a regional pollen signal caused by pollen dispersal over a wide area (Fægri and Iverson, 1964), most sites have a localised pollen “fingerprint” superimposed upon the regional signal. The study of pollen grains by palynologists is, therefore, widely used to reconstruct past landscapes and quantify environmental change (e.g. McCarroll et al., 2015; Webb et al, 2017). In addition to fossilised pollen being used to understand historical temporal change of specific sites, pollen profiling can also be used to answer contemporary spatial questions. One of the most novel modern-day palynological applications involves analysis of pollen attached to, or embedded within, items connected to a crime.

Since the 1950s, forensic palynology has enabled evidence from pollen and other plant palynomorphs (such as spores associated with plant reproduction) to assist criminal investigation and secure convictions around the world (Horrocks & Walsh, 1998; Wiltshire, 2006a; Bull et al., 2006a). Palynological investigation can involve examining items potentially associated with crimes as diverse as forgery, drug dealing, robbery, terrorism, assault, rape, and homicide (Mildenhall, 1990; Milne et al., 2004). Analysis of fabric – including clothing, shoes, and materials such as blankets and carpet – is particularly common in forensic settings. The insights that can be provided by studying fabric include: (1) matching clothing from a suspect to a crime scene; (2) locating proceeds of crime or human remains; (3) analysing clothes of a murder victim to establish peri-mortem fate and to differentiate the scene of a crime from the scene of deposition; and (4) providing evidence to support or refute alibis (Mildenhall et al., 2006).

In many cases where palynological evidence is used to help solve a crime or secure a conviction, the adhesion of pollen on fabric, and its subsequent retention on that fabric, is critical. In the case of clothing, pollen can be transferred directly (e.g. by brushing up against vegetation: Mildenhall, 2006a) or indirectly (e.g. via contamination by soil or dust: Monckton-Smith et al., 2013). Following adhesion, pollen can be lost from clothing as a result of everyday activities, including being rubbed or shaken off during wear, generating a natural decay curve. Alternatively pollen can remain on the surface of the fabric or become embedded within the weave (Bull et al,. 2006b). Ensuring that palynological evidence is robust requires an understanding of the factors that influence adhesion and retention of pollen (Wiltshire, 2006b). This is vital to ensure that simplistic assumptions about how pollen (of different species) is transferred to and from clothing (of different fabric types) does not lead to incorrect inferences being made by expert witnesses, which could affect testimony and verdicts in courts of law.

Understanding the complexities of pollen-clothing interactions requires an awareness of the biology of a pollen grain. In general terms, the lack of mobility of individual plants is mitigated by production of small, light, and highly-mobile pollen grains. The mobility of pollen grains en route to their intended destination (the stigma of the flower of an adult plant of same species) relies on one of four vectors to transport the individual grains: the wind (anemophily); insects (entomophily); mammals and birds (zoophily) or water (hydrophily) (Faegri and Van Der Pijl, 2013). The latter two pollination vectors are uncommon for angiosperms (Philbrick,1991; Regan et al., 2015). Following vector transfer, an important factor determining eventual reproductive success is the adhesion of the pollen grains to the recipient stigma. Adhesion efficiency can be increased by the presence of pollinkitt, a viscous liquid coating the exine (outer layer) of pollen grains (Pacini & Hesse, 2005; Lin et al., 2013). Pollenkitt is present, in varying quantities, on fresh pollen of all species, but is likely to be most advantageous when an insect vector is involved (Lin et al., 2013). From a forensic perspective, it is important to note that pollenkitt degrades with time. Thus it is possible that fresh pollen with abundant pollenkitt may adhere to, and be retained on, clothing or other exhibits to a greater extent than older, dehydrated pollen of the same plant species (even if this is still from the same season).

Pollenkitt is not the only factor that influences the adhesion and retention of pollen grains to various substrates. Firstly, the morphology of the exine is vital. Pollen grains differ markedly in their ornamentation and sculpturing, and this can link to pollination vector mechanism (Grayum, 1986; Mizes, 1995; Tanaka et al., 2004). For example, smooth pollen grains tend to be associated with wind-pollinated species while sculptured pollen grains, which might be more likely to adhere to fabric and less susceptible to subsequent removal, tend to be associated with plant species that rely on insect or animal vectors (Tanaka et al., 2004; Lumaga et al., 2006; Sannier et al., 2009). Secondly, the size of the grain can be important, especially in determining how likely a pollen grain is to embed within the fabric (Bull et al., 2006b). Thirdly, the type of fabric might be important, with the weave or electrostatic forces playing a role in pollen attraction and retention (Martin, 1940; Chen, 2013). Finally, the intensity of wear after pollen adhesion could affect the proportion retained. This is an important factor when assessing the natural decay curve of pollen from clothing that will be utilised in a forensic investigation.

In addition to understanding the factors that affect pollen adhesion and (non-deliberate) loss of pollen from fabrics before they reach a forensic laboratory, it is also important to quantify the efficacy of standard laboratory protocols to deliberately remove pollen. It is vital that pollen adhering to pertinent objects in a criminal case is removed in a systematic, thorough, efficient and standardised manner. It is not possible to efficiently assess an entire garment to count and identify pollen in situ, so pollen must be removed for assessment under a light microscope. Often, due to the destructive method of retrieval, palynology is the last in a line of examinations undertaken by forensics teams (Horrocks, 2004). Attempts have been made to retrieve pollen in several different ways, for example by: (1) refluxing small pieces of fabric in potassium hydroxide (Horrocks, 2004), (2) using sticky-backed tape or roller (Flinn, 1992), (3) using a vacuum (Jantunen and Saarinen, 2011), and (4) via hand washing retrieval techniques (Wiltshire, 2016). Retrieval rates are variable (Adams-Groom, 2012), and despite some methods being quick and easy, such as using tape (Flinn, 1992), hand washing retrieval techniques are most efficient (Bull et al 2006b; Wu et al., 2006; Zavada et al, 2007; Jantunen and Saarinen, 2011). It is vital that forensic retrieval techniques remove a representative sample of the pollen assemblage on the clothing, even if they do not remove all grains. Retrieval techniques need to be thorough enough to retrieve stubbornly attached pollen grains which may be pertinent to a case, particularly as pollen can still be recovered using hand washing techniques after items have been washed in a mechanical washing machine (with household detergent) and after commercial dry cleaning. (Bull et al, 2006b; Pers. Obs.)

Although taphonomic variability of pollen (i.e. variability in the fate of pollen between leaving the parent plant and recovery in forensic laboratories) has been studied previously, there are several major gaps in knowledge. Wiltshire (2006b) highlights the importance of analysing variables rather than assuming knowledge, however, to date, most studies have focussed upon simple description of the relationship between pollen and fabric rather than quantifying the drivers responsible for those trends. Here, we build upon the work done by Bull et al (2006b) and Boi (2015). The former studied pollen loss on different fabrics over time and found substantial differences, while the latter examined loss of pollen of different species from different fabrics but without a robust baseline from which to derive accurate retention data. These studies did not investigate the mechanisms driving the patterns they described, such that the potential effects of taphonomic variables including pollen morphology and wear intensity, were not examined. Moreover, neither Bull et al (2006b) or Boi (2015) examined the efficacy of laboratory retrieval techniques. Seemingly the only study to empirically test laboratory retrieval is that of Jantunen and Saarinen (2011) who examined taping and vacuuming protocols, but not hand washing retrieval procedures. There has seemingly been no comprehensive, holistic study of the causal mechanisms behind taphonomic variability of pollen both before clothing arrives at a forensic laboratory and during processing at such a facility. Such research would be helpful in assisting forensic palynologists, who are engaged in the complexities of interpreting palynological data as part of a forensic reconstruction, by providing an insight into multifaceted variability of pollen retention.

This study seeks to establish relative amount of pollen retained on clothing after a period of simulated light or heavy wear based on characteristics of the pollen (fresh versus dried; pollen morphology; pollen grain size) and on characteristics of the clothes: (fabric type). The specific hypotheses being tested are: (1) fresh pollen will be retained on clothing more readily than dried pollen because of the action of pollenkitt; (2) pollen retention will be lower on fabric with (simulated) heavy wear than (simulated) light wear; (3) pollen from insect-pollinated species will be retained on clothing more readily than pollen from wind-pollinated species because of its surface morphology; (4) small pollen grains will be retained on clothing more readily than large pollen grains as they are likely to embed deeper into the weave of clothing; and (5) pollen grains will be retained at different concentrations on different fabric types based on the weave of the material. We also test whether standard laboratory procedures to retrieve pollen by washing are equally effective regardless of species and fabric. Based on the results, we highlight key limitations of using palynological evidence within forensic settings and make recommendations on the importance of understanding pollen and fabric characteristics.

Materials and Methods

Laboratory methods

To explore potential variation in adherence and retention of pollen on fabric, pollen was transferred directly from the flower anthers onto a microscope slide under laboratory conditions. Eight different species were studied: grass (Poaceae), nettle (*Urtica dioca*), ribwort plantain (*Plantago lanceolata*), common lime (*Tilia x europaea*), Scots pine (*Pinus sylvestris*), catnip (*Nepeta cataria*), common daisy (*Bellis perennis*), dandelion (*Taraxacum officinale*). These species were selected to provide a range of grain sizes (18 – 65μm), variation in surface structure and ornamentation, and to represent both wind- and insect-pollinated species (Table 1). In all cases, 500 ± 30 grains were precisely counted on the specific microscope slide; any adjustments needed were made. Grains were then transferred from the slide to a piece of test fabric (8.5 cm x 3 cm) by smoothing the slide across a predetermined and pre-marked target area (1cm^2). The slide was checked under an optical microscope to ensure all grains had been transferred and the transfer process was repeated if necessary. To explore pollen retention on a range of different weave types, three different test fabrics were used: cotton, denim and fleece (photographs from Scanning Electron Microscope (SEM) shown in Fig. 1 for illustrative purposes).

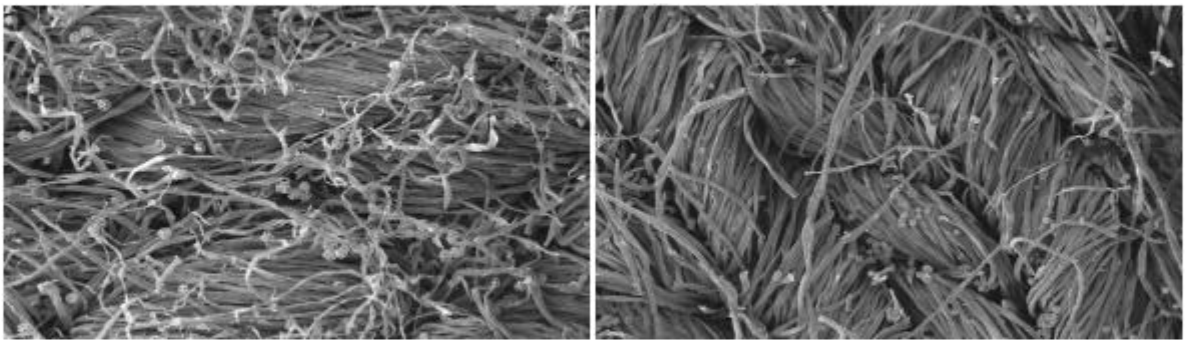


Figure 1 SEM image at x 200 magnification demonstrating how imbedded individual pollen grains can become in the weave of different fabrics (left: dandelion on denim; right: daisy on cotton).

To mimic a person acquiring pollen on their clothes directly by brushing past vegetation, fresh pollen (i.e. that collected from the anthers of freshly-collected plants) was used. This pollen was still influenced by a coating of pollenkitt. Six replicates were undertaken for each species/fabric combination. Then, to mimic a person acquiring pollen indirectly from soil or dust, six further replicates were undertaken for each species/fabric combination using pollen from plants that had been allowed to dry in paper envelopes. In these samples, the pollenkitt had dehydrated. This provided a total sample size of 288 (8 species * 3 fabrics * 12 replicates). Pollen grains were collected from anthers at various stages of development to ensure grains of different maturity were captured. Pollen was not acetelised or dehydrated in alcohol as this causes damage to the pollenkitt, which might affect adherence relative to natural conditions (Hesse, 1989).

Once all test fabric samples had been prepared, they were split equally between two wear intensity groups to mimic the natural agitation that clothing undergoes whilst being worn. To simulate light wear following pollen acquisition, 50% of test fabric samples were shaken for one minute on a mechanical shaker (500 oscillations per minute). To simulate heavier wear, the test fabric was rubbed 20 times with a clean piece of cotton, mimicking brushing of clothing or contact with upholstered surfaces. Cotton was selected because of its neutral electrostatic charge. In all cases, the number of pollen grains remaining on the test fabric after treatment was counted under the microscope. The number (and percentage) of grains retained relative to the number originally placed on that particular piece of test fabric was then ascertained.

Finally, all fabric pieces were washed in mild surfactant (Multi Purpose Detergent, Teepol, Orpington, UK) to retrieve the remaining pollen from the fabric. As noted above, this process is the standard recommended laboratory protocol for the retrieval of pollen grains from clothing (Wiltshire et al 2015; Wiltshire, 2016). The number of residual pollen grains on the fabric after washing was assessed by counting under the microscope. The efficiency of the washing technique was quantified by dividing the number of pollen grains remaining after washing by the number on the test fabric after simulated wear. The reciprocal was then taken and multiplied by 100 to give washing efficiency expressed as a percentage (e.g 80% washing efficiency indicated that 80% of pollen grains were removed after simulated wear by the washing action). All 288 test fabric pieces were washed in this way. Washing was always done after simulated wear rather than after the pollen transfer step in order to make the timeline of forensic analysis realistic (i.e. clothing delivered to laboratories for processing would typically have been worn after initial pollen transfer).

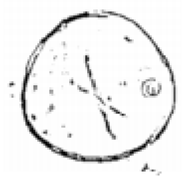
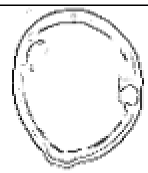
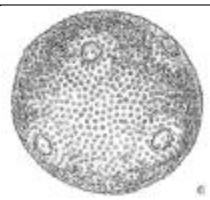
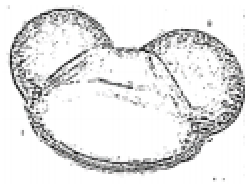
Statistical analysis

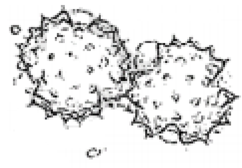
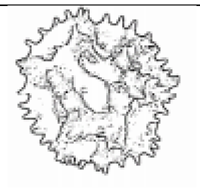
The first analytical step was to compare pollen retention rates for fresh versus dried pollen both overall and for each individual species using independent samples t-tests. This was done on the basis that this not only tested the first research hypothesis, but would also allow data from fresh and dried samples to be pooled in the event of no statistical differences being found. The same approach was used to test for any differences in pollen retention according to wear intensity.

To analyse the effects of pollen and fabric characteristics on pollen retention after light wear, a range of General Linear Models (GLMs) was constructed. Specifically, univariate models were created to analyse the effects of pollination type (insect-pollinated versus wind-pollinated), pollen grain size, and fabric type (cotton, denim, fleece) in isolation. Ultimately, a fourth univariate model was constructed based on species. Multivariate GLMs were then constructed for combinations of factors (1 = pollination type and fabric; 2 = grain size and fabric; 3 = pollination type, grain size and fabric; 4 = species and fabric). In all cases, all two-way interactions were also entered. To compare between the different competing models, Akaike's Information Criterion (AIC) was calculated using the formula AIC = -2 (log-likelihood) +2K, where K = the number of predictor variables. This allowed identification of the model that best balanced explanatory power (model fit) with parsimony (minimising the number of variables in the model). The model with the smallest AIC value (ρAIC = 0) was considered the optimum, with any other model within ρAIC ≤ 2 regarded as essentially having the same support as the optimum, models with ρAIC of 3-4 having strong support, models with ρAIC having 5-9 considerably less support, and models with AICρ ≥10 having essentially no support (Burnham and Anderson, 2002).

All analysis used raw data (pollen grains retained after simulated wear relative to those initially added), which were normally distributed. Graphical presentation of data used percentage for clarity. All analysis was undertaken in IBM SPSS (v22.0).

Table 1 Morphological characteristics of pollen types used in this study (Moore et al 1991). Sketched images derived from author's own photographs.

Species	Size (approximate)	Structure	Surface pattern (ornamentation)	Method of pollination	Illustration (not to scale)
Poaceae Grass	40 µm	Monoporate	Psilate	Wind	
Urtica dioca Common nettle	18 µm	Triporate	Scabrate	Wind	
Plantago lanceolate Ribwort plantain	35 µm	Periporate	Verrucate	Wind	
Tilia x europaea Common lime	45 µm	Triporate	Foveolate	Insect	
Pinus sylvestris Scots pine	65 µm (with sacs separate)	Saccate	Grain body psilate, sacs with irregular reticulum	Wind	
Nepeta cataria Catnip	35 µm	Polyzonocolpate	Eureticulate	Insect	

Bellis perennis Common daisy	35 μm	Inaperturate	Conical echinate	Insect	
Taraxacum officinale Dandelion	45 μm	Inaperturate	Echinate in ridges or crests	Insect	

<u>Results</u>

Fresh versus dried pollen

Pollen retention after simulated wear was 42.9% on average. However, contrary to prediction, there was no statistically significant difference in retention of fresh or dried pollen either for all species combined (fresh = 44.9% ± 2.4% SEM; dry = 41.0% ± 2.4% SEM; $t = 1.290$, d.f = 286, $p = 0.198$) or for any individual species ($p > 0.05$ in all cases; tests not shown). Accordingly, data from both fresh and dried replicates were pooled on a per-species basis to double the sample size and thus increase the robustness of subsequent analyses.

Wear intensity

As expected, there was a substantial difference in pollen retention according to wear intensity, with considerably more pollen being retained after light wear than after heavy wear (59.0% versus 26.5%, respectively, Fig. 2). This was statistically significant ($t = 11.637$, d.f = 286, $p <0.001$).

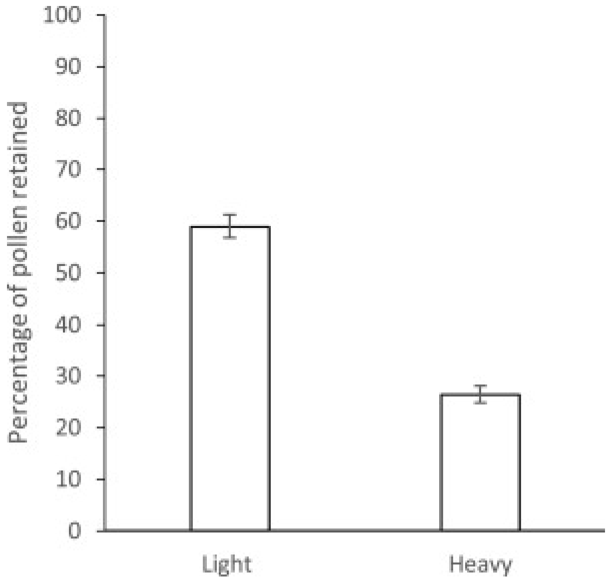


Figure 2 Pollen retention after light intensity and heavy intensity wear.

Pollen retention following wear: effect of pollen and fabric characteristics

As predicted, pollen from insect-pollinated species was retained at higher concentrations than pollen from wind-pollinated species (50.4% and 35.0%, respectively). This pattern was consistent regardless of wear intensity (Fig. 3a-b), but statistically pollination type explained more of the variability in pollen retention after light wear (R^2 = 0.175) than after heavy wear (R^2 = 0.035) (Table 2).

Contrary to expectation, there was no clear linear relationship between pollen size and its retention following wear (Fig. 3c-d). Despite this, retention was higher for some size categories (35 µm and 45µm) than others, such that size was a significant predictor of pollen retention for both light and heavy wear (R^2 = 0.188 and 0.073: Table 2). These non-linear patterns were consistent regardless of wear intensity, which suggested that findings might relate to underlying links to pollen sculpturing rather than size per se. This was borne out by echinate pollen occurring in the high retention groups (35 µm = daisy; 45 µm = dandelion), whereas species in the size classes with the lowest retention (18µm and 40) included nettle and grass, which, although different in their fine sculpturing, have a much smoother surface. Accordingly, a new analysis was undertaken whereby pollen retention was related directly to species. Species identity explained a high proportion of the variation in the retention of pollen for light and heavy wear (R^2 = 0.343 and 0.198 respectively), which was substantially higher than the variance explained by either pollination type or grain size (Table 2). The species retained in the highest concentrations were consistent regardless of wear intensity (dandelion and catnip) but species with the lowest retention differed (Fig. 3g-h). Very broadly though, pollen retention increased as the surface morphology became rougher or more echinate (i.e. following the order in which species are presented in Table 1 and Fig. 3g-h).

As expected, fabric type was significantly related to pollen retention regardless of wear intensity but the effect size differed substantially (light wear R^2 = 0.082; heavy wear R^2 = 0.259: Table 2). Following light wear, fleece retained less pollen than cotton and denim, which were approximately equal (Fig. 3e). Following heavy wear, pollen retention on fleece and cotton were approximately equal but retention on denim was substantially higher (Fig. 3f)

The optimal univariate model for predicting pollen retention after light wear was based on plant species (ρAIC = 0 and R^2 = 0.343; no other models with ρAIC<10; Table 2). The model that explained the least variability (and had the highest ρAIC score) was that based on fabric type. This suggests that: (1) when clothing had only been lightly worn, pollen characteristics were more important than fabric characteristics in determining retention but also that: (2) this was highly species-specific such that pollination type and grain size were too crude to meaningfully predict retention. Conversely, the optimum univariate model for predicting pollen retention after heavy wear was based on fabric type (ρAIC = 0 and R^2 = 0.259; no other models with ρAIC<5; Table 2). This suggests that following heavy wear, fabric characteristics were more important than pollen characteristics in determining retention.

While it is instructive to examine pollen and fabric characteristics separately, in reality these factors co-occur and interact with one another. The optimal model for both light and heavy wear was that which combined both plant species and fabric type, as well as the interaction between them (ρAIC = 0; R^2 = 0.640; Table 2). As expected given the univariate results, the relative contribution of these factors to the overall model depends on wear intensity, with species being the larger contributing factor for light wear and fabric being the larger contributing factor for heavy wear. The other multivariate models all had ρAIC values >10 and substantially lower R^2 values. The complexity of the interaction between species and fabric in determining pollen retention following both light and heavy wear is shown in Fig. 4. For example, following light wear, almost all (>94%) dandelion pollen was retained on cotton and denim versus just 58% on fleece, while 90% of pine pollen was retained on denim versus 60% on cotton and 54% on fleece. Retention of pollen following heavy wear was 3always lower than following light wear, but interactions between species and fabric were similarly complex. Particularly substantial differences were evident for grass, which ranged from 50% retention on denim to just 7% on cotton and 2% on fleece, and lime, which was retained in approximately equal amounts on denim and fleece (~24%) versus 7% on cotton.

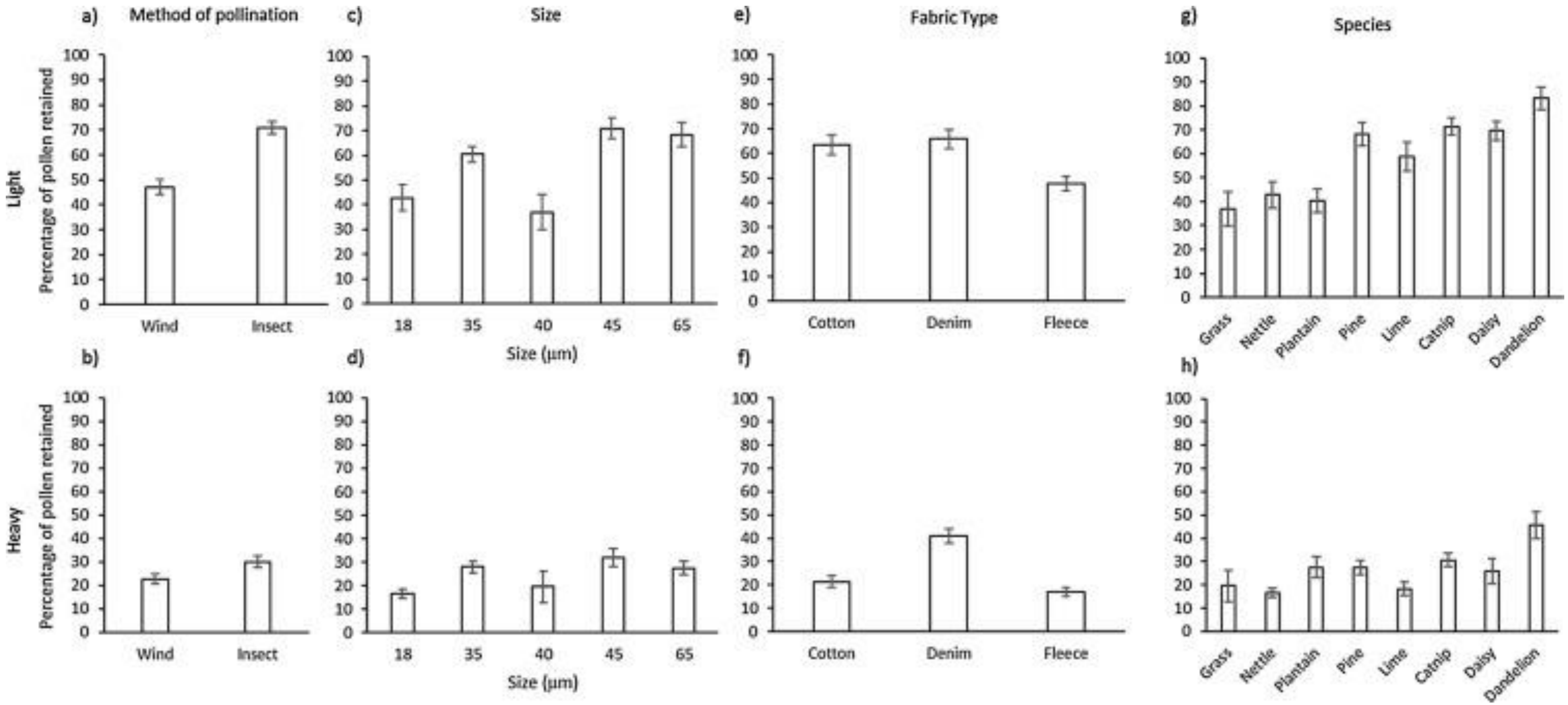


Figure 3 Pollen retention following wear and the effect of pollen type, size and fabric characteristics.

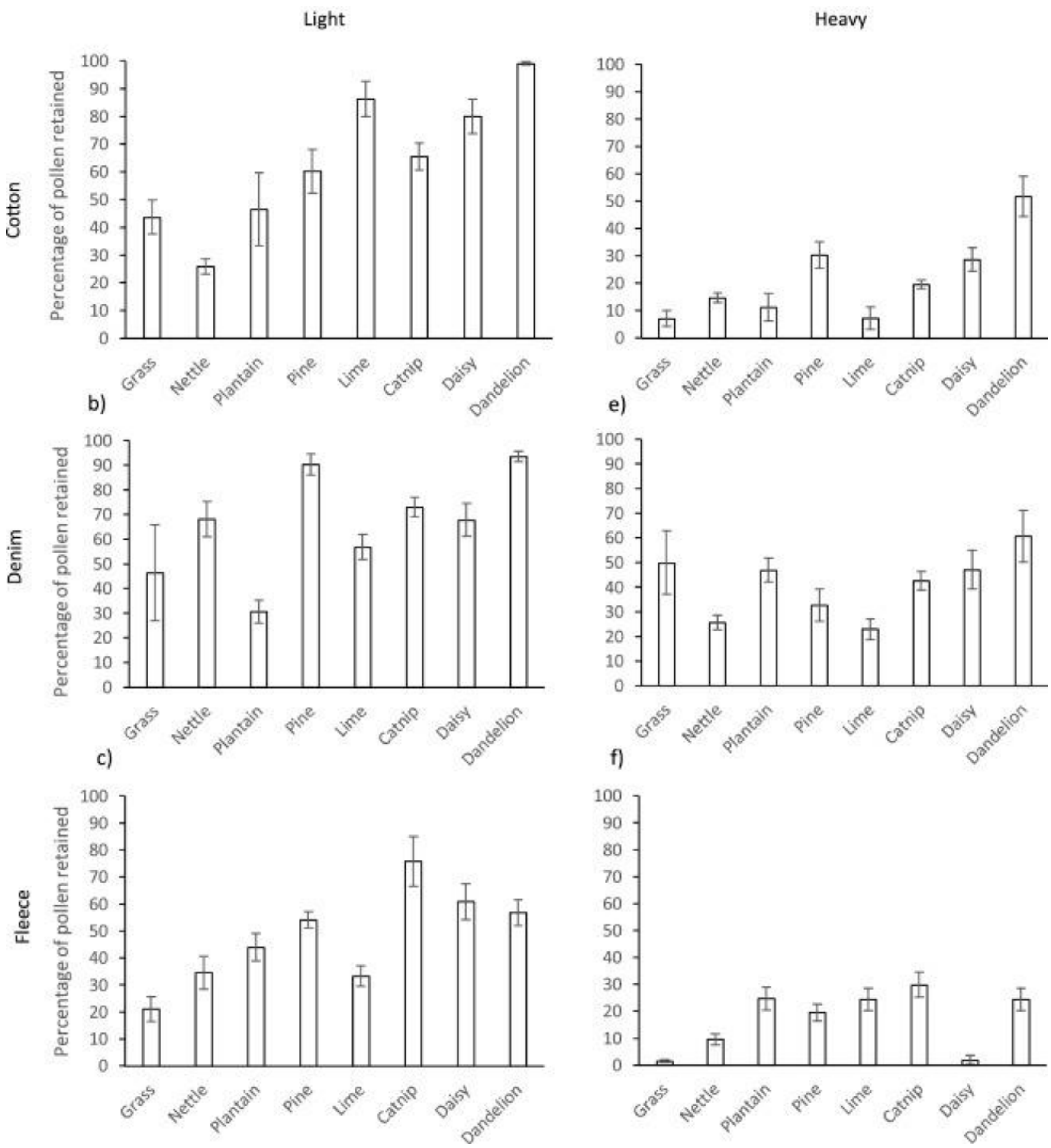


Figure 4 The complexity of the interaction between species and fabric in determining pollen retention following both light and heavy wear.

Table 2 General Linear Models of pollen retention on fabric following light or heavy wear according to pollen and fabric characteristics. Delta Akaike's Information Criterion scores are used to compare all models (ρAIC^1) and to compare univariate models only (ρAIC^2) as per the Methods.

		Light wear intensity							Heavy wear intensity					
		F	df	P	R^2	ρAIC^1	ρAIC^2		F	df	P	R^2	ρAIC^1	ρAIC^2
UNIVARIATE MODELS														
1	Pollination Type	27.552	1	<0.001	0.175	49.435	14.263		5.216	1	0.024	0.035	59.418	16.468
2	Size	7.352	4	<0.001	0.188	48.431	13.259		2.753	4	0.031	0.073	56.907	13.957
3	Species	9.254	7	<0.001	0.343	35.172	0		4.753	7	<0.001	0.198	47.9872	5.0372
4	Fabric	5.725	2	0.004	0.082	56.139	20.967		24.608	2	<0.001	0.259	42.95	0
MULTIVARIATE MODELS														
1	Pollination Type	31.535	1	<0.001					6.965	1	0.009			
	Fabric	7.615	2	0.001					25.43	2	<0.001			
	Pollination Type* Fabric	3.106	2	0.048					0.371	2	0.691			
	Overall	10.633	5	<0.001	0.297	41.442			11.713	5	<0.001	0.298	41.462	
2	Size	9.913	4	<0.001					3.995	4	0.004			
	Fabric	12.227	2	<0.001					23.048	2	<0.001			
	Size*Fabric	5.428	10	<0.001					2.05	10	0.045			
	Overall model	7.251	17	<0.001	0.465	24.392			6.336	17	<0.001	0.407	30.946	
3	Pollination Type	24.539	1	<0.001					0.08	1	0.777			
	Size	7.593	4	<0.001					2.102	4	0.084			
	Fabric	11.507	2	<0.001					18.944	2	<0.001			

	Pollination Type*Fabric	5.636	2	0.005					1.94	2	0.148			
	Size*Fabric	4.728	10	<0.001					2.447	10	0.017			
	Overall	8.517	20	<0.001	0.559	14.185			5.491	20	<0.001	0.426	31.01	
4	Species	13.179	7	<0.001					9.328	7	<0.001			
	Fabric	12.358	2	<0.001					42.973	2	<0.001			
	Species*Fabric	4.536	14	<0.001					4.352	14	<0.001			
	Overall	8.257	23	<0.001	0.637	0			9.225	23	<0.001	0.639	0	

Effectiveness of laboratory pollen removal techniques

Surprisingly, efficiency of washing was lower than expected: only 67.7% of pollen was removed following standard laboratory protocols, rather than the 100% often assumed. Moreover, this overall mean percentage was subject to considerable variation based on previous wear intensity (78.5% versus 58.9% washing efficiency following light and heavy wear, respectively). Even more concerning, the efficiency of this washing protocol differed very substantially according to plant species, fabric type, and the interaction between these factors (Table 3). These interactions are shown in Fig. 5. Particularly striking are the differences between washing efficiency for dandelion on all fabrics after both light wear (mean = 97%) and heavy wear (87%) versus the very low efficiency for nettle (39% and 45%, respectively). Generally, pollen retention followed a similar species pattern on all fabrics following light wear, although the efficiency of washing for retrieval of lime pollen varied considerably according to fabric type, both in absolute terms and relative to the other species (Fig. 5 330 a-c). The efficiency of washing following heavy wear was much more variable according to both species and fabric; the results of grass (high retrieval on denim, low on cotton and fleece) and plantain (high retrieval on cotton, low on denim and fleece) are particularly notable in this regard (Fig. 5 d-f).

Table 3 General Linear Models of pollen retention on fabric after hand washing revival according to plant species and fabric type.

	Light wear intensity					Heavy wear intensity			
	F	df	P	R^2		F	df	P	R^2
Fabric	9.948	3	<0.001			12.149	2	<0.001	
Species	30.449	7	<0.001			13.54	7	<0.001	
Fabric*Species	6.98	14	<0.001			6.365	14	<0.001	
Overall	14.28	23	<0.001	0.681		9.811	23	<0.001	0.575

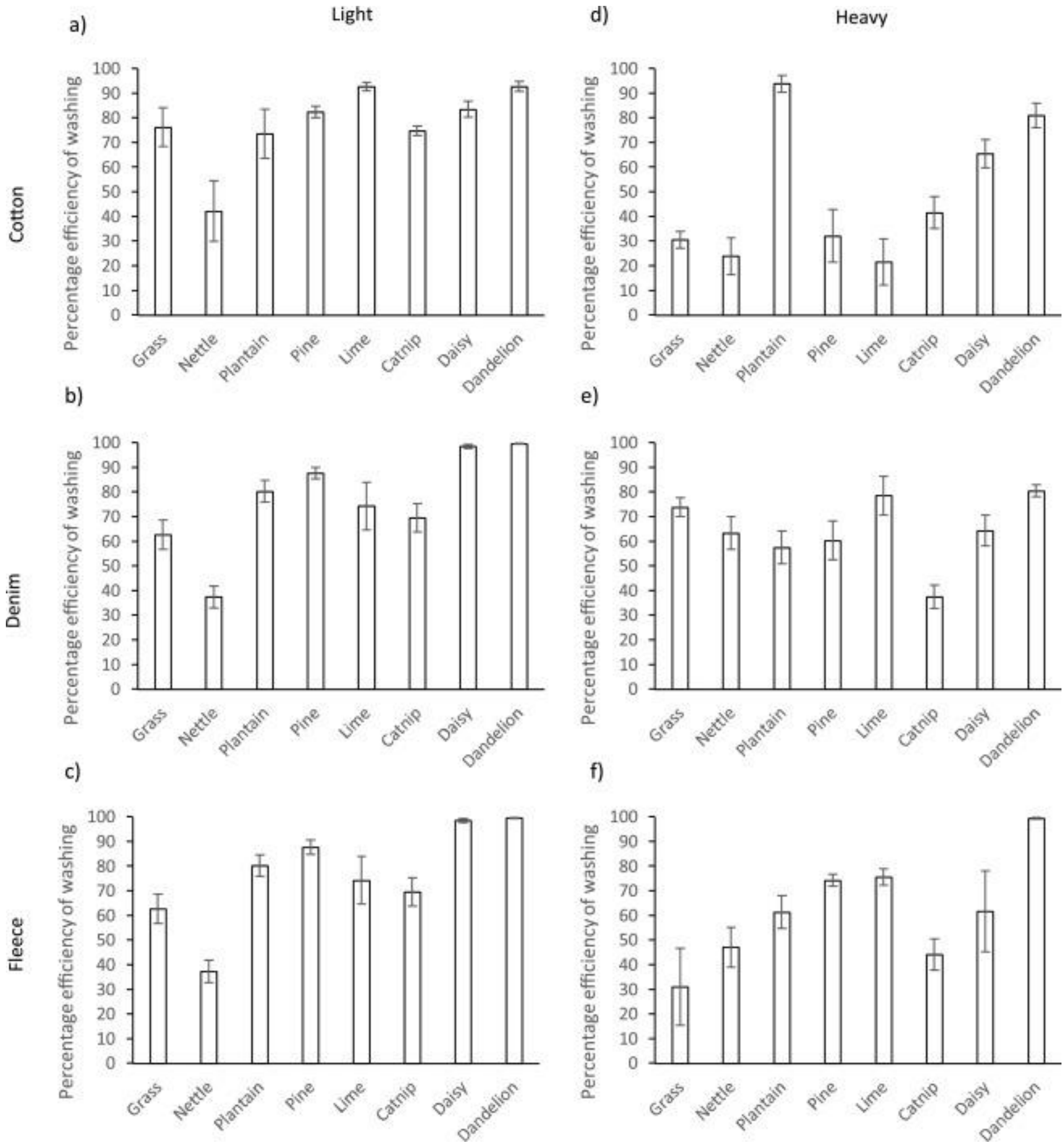


Figure 5 Efficiency of handwashing using standard laboratory protocols for retrieving forensic parynology evidence. The washing efficiency was quantified by dividing the number of pollen grains remaining post-washing by the number on the test fabric after simulated wear. The reciprocal was then taken and multiplied by 100 to give washing efficiency expressed as a percentage.

Discussion

As with most types of forensic evidence, the timing of palynological analysis is critical. Given that almost 60% of the pollen that adheres to clothing is lost through subsequent wear, pertinent objects need to be seized quickly if palynology is likely to be important in forensic reconstruction. This study has also shown that loss versus retention of pollen on clothing varies substantially according to pollen characteristics *and* fabric type. As such, differential loss of pollen of different species during normal activity could mean that the pollen assemblage found during analysis does not fully align with the plant assemblage encountered at the scene. This might be of particular importance in cases where the absence of a specific species in a garment's palynological profile can be explained by taphonomic variables. For example, in a case where an accused assailant flees a scene of crime through an area of flowering grasses, it might be assumed that analysis of trouser material below the knee should reveal abundant grass pollen. However, our results show that grass pollen is easily lost from cotton garments during running or walking, or by simply brushing the area that has been in contact with the flowers, such that the absence of grass pollen might not be surprising. It is also vital to understand the effect of wear intensity on pollen retention, since this not only affects the amount of pollen that is retained overall, but also the relative proportion of different species that will be found in forensic analyses.

The importance of pollen characteristics

Pollen characteristics affect retention on all fabric types, and at both high and low wear intensities. Surprisingly however, and contrary to our hypothesis, general patterns between pollen retention and simple categories such as pollination type or grain size were comparatively weak. For example, although pollen from insect-pollinated species was retained at higher levels than pollen from wind pollinated species overall, and this was statistically significant, this finding was too variable to be particularly helpful in palynological interpretation (as demonstrated by the low R^2 values, especially in comparison to models using full species information (Table 2)). Similarly, although grain size was also a significant predictor of pollen retention, this was not a linear trend as suggested by Bull et al (2006b). Thus, there was no generally-applicable pattern that, for example, smaller grains are always likely to be retained more readily, and at higher levels, than larger grains. The lack of the generality of findings can also be applied, to a large extent, to pollen sculpturing. For example, given the high ornamentation of dandelion and daisy grains (Table 1), it is unusual for them not to share more similar retention properties (Mizes, 1995).

The models that were most useful in explaining variability in retention all included species identity, rather than general pollen characteristic variables. This shows that pollen retention is highly species-specific and underlines the complexity of pollen:clothing relationships. Accordingly a forensic palynologist should be careful in inferring likely retention of pollen from one specific species based on data from another species - even one pollinated in the same way, of a similar size, and of similar sculpturing. As such, detailed understanding would require species-specific empirical testing rather than application of general principles from other experimental data.

One general finding that does seem to hold, though, is that the age of the pollen does not affect retention and should not confound palynological forensic evidence. This is despite the fact that fresh pollen is characterised by the presence of pollenkitt, a viscous liquid coating the exine, which can affect adhesion (Pacini and Hesse, 2005; Lin et al, 2013). Our finding that there is no difference in retention of pollen according to whether it is fresh or dried suggests that pollenkitt is less important than previously thought in terms of adherence to, and retention on, clothing. This means that in a case where an accused assailant has brushed against flowering vegetation and/or come into contact with dried dusty surfaces, the prosecution and defence teams can be assured that there is no differential loss of pollen based on pollen state.

The importance of fabric characteristics

Alongside the influence of pollen characteristics, fabric type is extremely important in explaining pollen retention, especially following heavy wear. This is in contrast to Zavada et al (2007) who found fabric type did not affect the amount of pollen that adhered to clothing from natural pollen rain. Even more importantly from a forensic evidence perspective, our findings show that there is a significant interaction between species and fabric type, which extends the descriptive findings of Bull et al (2006b) and Boi (2015). For example, 86% of lime pollen was retained on cotton fabric versus only 33% on fleece, while 68% of nettle pollen was retained on denim versus 25% on cotton. Such findings mean that, as with pollen characteristics, there are few if any “rules of thumb” for fabric characteristics that can be used by forensic palynologists to predict the likely retention level for any given fabric. As such, the gold standard in palynological evidence would involve simulations like those presented in this paper to model retention for the pollen type in question on the same fabric worn by the accused to provide a baseline for comparison with actual exhibits. However, this would add substantially to the cost of forensic investigations and the time needed to prepare evidence, possibly prohibitively so.

One such case where a simulation of pollen retention might have been useful is a sexual assault case in New Zealand reported by Mildenhall (1990), where more pollen was discovered on the thighs of the victim's denim jeans than the knees. This might suggest that weave depth has an influence on the amount of pollen retained on a garment, as suggested in our data by the difference in retention between cotton and denim fabrics where weave depth is different. In Mildenhall's (1990) case the weave depth is due to the age and state of the jeans where the knees have become worn down over time. This therefore suggests that, in addition to fabric type being important, the age and condition of the fabric might also have an effect and needs to be factored into any simulations.

In addition to weave, the differential retention across fabric types could be influenced by their electrostatic state, relative to that of pollen grains (Martin, 1940; Chen 2013). There has been very little research conducted in this area. Vercoulen et al (1992) assessed the electrostatic charge of birch (*Betula*) and cocksfoot grass (*Dactyli*s), and finds that they are weakly negatively charged, but it is possible that different species might have different electrostatic states. Cotton is relatively neutrally charged, so would attract negatively-charged pollen grains (Vercoulen et al 1992), whereas polyester fleece is negatively charged and so would repel oppositely charged pollen grains. If pollen grains have different charge states, and given that different fabrics themselves have different charges, this might also partly explain the species:fabric interactions. For example, catnip pollen may be more positively charged and so fleece may have retained more of the pollen than other fabrics due to the electrostatic attraction. Talaty et al (2008) have previously highlighted the importance of understanding relative electrostatic forces when interpreting forensic evidence, in relation to negatively charged explosives ions in relation to fleece fabric. Further study on possible differences in the charge of pollen grains of different species, and in relation to different fabrics, is recommended as an area for future research.

Effectiveness of laboratory pollen removal techniques

Laboratory pollen harvesting from clothing or fabrics is undertaken on the basis that it should be almost 100% efficient (i.e. remove all pollen grains remaining on the fabric upon receipt of the clothing for processing), or at least remove a representative sample. It has previously been reported that handwashing items is the most efficient method of pollen retrieval (Bull et al 2006b; Wu et al., 2006; Zavada et al, 2007), but has not been included in previous research on methods of retrieval of pollen from fabrics (Jantunen and Saarinen, 2011). However, our study on hand washing retrieval has shown that across the pollen types and fabrics used here there is great variability (range of 21 – 93% efficiency on cotton fabric between species). Palynological interpretation must only be undertaken with extreme caution as this study shows that the commonly used approach for retrieving pollen from a garment, might not yield a representative assemblage. For example, analysis of clothing from a suspect accused of being at a crime scene characterised by abundant dandelion could be misleading. At face value, if high levels of dandelion were retrieved during forensic investigation, this would be strong circumstantial evidence placing the suspect at the crime scene. However, given that results here show that dandelion is extremely well retained on fabric following wear but also extremely easy to remove via laboratory washing retrieval, a defence barrister might argue that the residual dandelion pollen on the suspect's clothes is present from historical (and innocent) movements, rather than being an indicator that the suspect has been to the crime scene.

Moving forwards: conclusions and recommendations

One potential way forward here is to utilise Bayesian techniques as palynological evidence is often assessed using Bayes' Theorem. Indeed, Horrocks and Walsh (1998) discuss the importance of insuring that the pollen profile from a suspect's clothing matches that to a crime scene and outlines a likelihood ratios framework for assessing the extend of that match and its likely significance. However, although this takes into account unequal abundance of different species, it does not account for potential differential retention of the species on the fabrics. One the strengths of a Bayesian approach is the ability to weight different lines of evidence in a model (NRC, 2009; Lund and Iyer, 2017). In theory therefore, it would be possible to use general trends relating to pollen or fabric characteristics, as well as important taphonomic variables (such as pollen productivity) to improve the efficiency of the model in characterising the strength of the evidence. In reality, the findings here demonstrate that pollen retention patterns are so complex, and so species specific, it would be difficult to rigidly apply a set of general principles in this way. However, results do suggest that focussing primarily on pollen of insect-pollinated species in forensic palynological investigation may be advantageous.

Forensic palynology is an important field within forensic analysis. However, to ensure that such evidence is maximally useful, it is important to ensure interpretation is based upon empirical simulations rather than being predicated upon simplistic assumptions about how pollen (of different species) is transferred to and from clothing (of different fabric types). This is vital to ensure that incorrect inferences do not confound evidence given by expert witnesses, and thus affect testimony and verdicts in courts of law.

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