Are your hands clean? Pollen retention on the human hand after washing

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Keywords

Pollen; Human skin; Hand washing; Forensic Palynology; Taphonomy; WHO Hand Hygiene Technique

Highlights

Palynology can link people or objects to localities with distinctive vegetation

Study of pollen retention on human skin through hand-washing using WHO guidelines

A mean of 0.93% (range 0.36-2.74%) retention through one hand-wash procedure

Trace amounts of several species survived multiple hand-wash procedures

Suspects' skin pollen load may be evidential even after hand-washing

Abstract

Pollen retention on clothes, footwear, hair and body has been used to link people to localities with distinctive vegetation, or soils containing distinctive palynomorphs. Little attention has been given to human skin as a possible medium for carrying a forensically-important pollen load and whether this might survive attempts to remove it. We report here the results of experiments testing the retention of pollen of ten flowering plant species on the human skin through repeated cycles of washing and drying hands, using the WHO protocol to standardise hand-washing and drying. Between 0.36% and 2.74% (mean 0.93%) of the initial pollen load was retained through a single hand-wash. Trace amounts of some species survived multiple hand-wash cycles. It is concluded that forensic analyses can be made of the pollen load of those parts of the skin that may have been in contact with palynologically-distinctive vegetation, even in cases where the person involved has washed, or been washed. These observations may also be of relevance in cases where human skin became contaminated with other microscopic particulates.

Introduction

Palynology is increasingly used as a Forensic Science technique, since pollen retained on persons or objects may link them to areas of distinctive vegetation, or soils containing a distinctive palynomorph load (e.g. Horrocks and Walsh 1998; Mildenhall 2006b; Bryant and Bryant 2019). It may also throw light on materials ingested before death (Mildenhall et al. 2006; Wiltshire 2009; Wiltshire et al. 2015),

even of a 5200-year-old mummy preserved in a glacier (Oeggl et al. 2007). Pollen of forensic significance may be retained upon footwear, clothing, hair or even in the respiratory tract and other internal parts of the body (e.g. Bull et al. 2006; Mildenhall 2006a, b; Wiltshire 2006; Wiltshire and Black 2006; Morgan et al. 2010; Wiltshire et al. 2015; Webb et al. 2018; Bryant and Bryant 2019). Pollen on the skin has been noted infrequently (e.g. Montali et al. 2006; Wiltshire 2009; Piotrowska-Weryszko et al. 2017).

Although pollen on clothing may survive hand- and machine-washing (Bull et al. 2006) or dry cleaning (Mildenhall 2006a), we are unaware of any literature assessing the effect of washing on the retention of pollen on human skin. Bacterial flora on the hands are known to survive brief washing (e.g. Noskin et al. 1995; Kac et al. 2005), so there is a possibility that other particulates, including pollen, will also survive. In this paper, therefore, we assess the potential for pollen to survive hand-washing, to ascertain whether palynological investigation of human skin may yield viable forensic information.

Materials and methods

Throughout the research, in order to standardise experiments as much as possible, hand-washing used the hand-wash and hand-drying protocol stipulated by the World Health Organisation (Clean Care is Safer Care Team 2009: Fig II.2; World Health Organisation 2020 [hereafter the 'WHO Protocol']). The work was done in three phases, a pilot study and then two episodes of quantitative research. In the pilot, non-quantitative study, the aim was to test if pollen would survive on the hands through several washes.

Pilot study

In this study, daffodil (*Narcissus pseudonarcissus* L.) coronas were removed and the exposed stamens of one flower were brushed across the back of the hand of a researcher, leaving a deposit of pollen grains visible to the naked eye (Fig. 1). The experiment was repeated four times, with different numbers of washes using the WHO Protocol.

1. No hand wash (control)
2. One hand wash and drying cycle
3. Two hand wash and drying cycles
4. Three hand wash and drying cycles

After the final wash and drying cycle of each test, the hands were rinsed with a jet of filtered water, with all rinse-water caught in a cleaned plastic bowl. The rinse-water was then passed through nominal 6 µm nylon mesh. Sieving on 7 µm micromesh has been demonstrated to lose only 0.4% of pollen grains (Cwynar et al. 1979). The retained fraction was stained with safranine and an aliquot was mounted on microscope slides in Aquatex mountant by the other researcher. Microscopic examination of the slides was at 100x and pollen was identified at 400x magnification.

Daffodil pollen was recovered from all four experiments. Two quantitative studies were therefore designed to explore this phenomenon further and test whether pollen retention through hand-wash was specific to daffodil pollen, or was part of a wider phenomenon.

Insert Fig. 1 here

Quality control for the second and third studies

In the work comprising the second and third experiments, quality control was enforced through careful cleaning of the researcher's hands and all surfaces before each experiment and by monitoring of air and water-borne pollen. The hands of the researcher were initially washed using the WHO Protocol with two pumps of Jangro Premium Bactericidal Hand Soap BK170-50 (about 3 ml, as recommended by the manufacturer), rinsed for 20 seconds in running water and dried using a paper towel before application of material from the target plant.

During the duration of each of these experiments, a slide made sticky with a thin film of petroleum gel was exposed adjacent to the wash station to monitor atmospheric pollen. At the end of each experiment a drop of stained Aquatex mountant was placed on a coverslip, which was then inverted onto the petroleum gel before the slide was examined microscopically.

Before each experiment started and after it finished, the tap water was run for 20 minutes through nominal 6 µm nylon mesh. The retained fraction was then mounted for microscopic examination using Aquatex mountant.

No pollen was recovered during these tests. The tap water was found to contain occasional plastic microfibres and very occasional roundworms. Very rare mineral dust was encountered in the atmospheric monitoring slides.

The second study

The second study involved three experiments, carried out one after another, on successive days. Overnight, between the experiments, the researcher took a shower and also washed his hands following food preparation, for hygienic purposes and other activities, such as an episode of gardening, which occurred between the second and third experiment. The number of these washes was not recorded but it may be noted that some were less rigorous than the WHO Protocol. The first experiment used flowers of daffodil; the second used flowers of tulip (*Tulipa* x*gesneriana* L.); the third used flowers of false Christmas cactus *Schlumbergera truncata* (Haw.) Moran (Fig. 2, Table 1). The daffodil and tulip flowers were sourced from a supermarket; the false Christmas cactus flower used in the study was the last flower produced by a houseplant at the end of it's flowering season.

Stamens of one flower were dissected out and applied to the hands of the researcher by rubbing gently against the back of the left hand using the palm of the right hand for 10 seconds, timed using a stopwatch. The researcher then washed his hands using the hand-wash and hand-drying following the WHO Protocol with the wetting of the hands, application of soap (3 ml Jangro Premium Bactericidal Hand Soap BK170-50) and vigorous rubbing of hands in the prescribed manner, timed at 20 seconds using a stopwatch, with a further 20 seconds of rinsing under running water. Drying of the hands with a paper towel was not timed. All wash-water used in the hand-wash and rinse was retained in a labelled clean plastic bowl. Four further hand-washing and drying cycles followed using the same WHO protocol and soap, with the wash-water for each retained in a separate labelled clean plastic bowl. A final hand-washing in an attempt to remove any remaining pollen used the WHO Protocol and soap, followed by careful scrubbing of all surfaces of the hand, especially in obvious crevices such as nail-beds, using a clean toothbrush under running water, before drying the hands with a paper towel (hereafter the 'WHO Protocol with scrubbing'). All wash-water from this procedure was also retained in a separate labelled clean plastic bowl. Therefore, each of the three experiments involved six hand-wash and drying cycles.

The wash-water retained in each bowl was then passed through nominal 6 µm nylon mesh and the retained fraction was placed in a graduated vial, which was topped up to 6 ml with filtered water. The vial was shaken briskly, then aliquots of 0.4 ml were withdrawn immediately using a graduated micropipette and placed on microscope slides. The aliquots were evaporated almost to dryness on a hotplate set to 95° C before a drop of Aquatex mountant was added and mixed with the aliquot using the corner of a coverslip, which was then placed on the mixture. Once the slides had cooled and the mountant had hardened, clear nail varnish was run around the edges of the coverslip to make the mounts permanent. Two aliquots from each sample were counted, with care being taken to space twelve traverses of the slide equally down the coverslip so that there was no overlap. Pollen was located using 100x magnification and identifications were verified using 400x magnification. It is estimated that this procedure covered 70% of the area of the coverslip.

During analysis of the material derived from the washing of hands following contamination of the hands with tulip pollen, it became apparent that pollen of daffodil was still being shed from the hands of the researcher, in spite of the careful scrubbing on the sixth wash of the first experiment. Following this observation, especial care was taken to avoid contact with plants used in the research during daily activities, to minimise the probability that hands were being re-contaminated inadvertently with pollen of these species. This special care was extended through the third experiment.

The third study

The protocol used in the second study was extremely time-consuming, which meant that only a very small selection of species could be analysed, given the resources available. It was therefore decided to abbreviate the procedure so that more taxa could be considered. The third study therefore consisted of seven experiments, using flowers of juneberry *Amelanchier alnifolia* (Nutt.) Nutt. ex M. Roem., thale cress *Arabidopsis thaliana* (L.) Heynh., Grecian windflower *Anemone blanda* Schott & Kotschy, marsh marigold *Caltha palustris* L., goat willow *Salix caprea* L., daisy *Bellis perennis* L. and Mexican orange *Choisya ternata* Kunth (Fig.2, Table 1). These were chosen because they were available to the researcher in his garden or in the lanes near his house, and to encompass as wide a range of plant families as possible.

Procedures were the same as in the second experiment, except that the WHO Protocol was used once, and this was followed by the WHO Protocol with scrubbing. Each of the seven experiments thus consisted of two hand-wash and drying cycles. The first four experiments were carried out sequentially on succeeding days and the last three experiments were carried out a week later on succeeding days. Between experiments the researcher took showers overnight and washed his hands following normal daily activities which included gardening. The number of hand-washes outside the experiment was not recorded, but many were likely to have been less rigorous than the WHO procedure. Care was taken to avoid plants previously used in the study, to avoid inadvertent contamination of the hands.

Insert Figure 2 here

Insert Table 1 here

Results

In the pilot study, pollen of daffodil was demonstrated still to be on the hands after washing and drying them three times following the WHO Protocol. This suggested that further experimentation and quantification was necessary.

The results of the second and third studies are shown in Tables 2 and 3 and Fig. 3. Table 2 gives the schedule of washes and shows the recovery of pollen from each protocol (wash). There were 32 washes in total for these experiments. Table 2 presents them in order - the first six washes follow contamination of the hands with pollen of daffodil, then the next six washes follow contamination of the hands with pollen of tulip and so on. It must be noted that the large pollen grains of daffodil, tulip and false Christmas cactus were retained in small numbers on the hands through multiple WHO Protocols and WHO Protocols with scrubbing; daffodil surviving at least 25 cycles, tulip at least 19 and false Christmas cactus at least 15, but seem to have been finally eliminated by the 26th, 20th and 16th washes respectively, since no further grains of these species were found during later experiments.

The smaller grains of the juneberry, thale cress and Greek windflower seem not to have been retained past the WHO Protocol with scrubbing following the initial WHO Protocol. Similarly-sized grains of marsh marigold and goat willow, however, survived five and three washes, before being eliminated by the sixth and fourth washes respectively.

It can be noted that for daffodil, tulip, false Christmas cactus, marsh marigold and goat willow there is a general, but uneven decay in numbers recovered for each species as hand-wash cycles progressed up to the 5th wash for these species (Fig. 3). Recovery of pollen of the first three species from further protocols was uneven.

Insert Table 2 here

Table 3 shows the number of pollen grains recovered in the initial WHO protocol and the total numbers of pollen retained through that protocol and later recovered. It also shows the percentage of the total pollen recovered that was retained through the initial WHO protocol (the initial wash). The mean percentage retained on the skin through the first WHO protocol was calculated as 0.93% of the total pollen recovery for all species.

Insert Table 3 here

Other materials and pollen grains were also seen, but not systematically logged during pollen counting. These include microplastic and other textile fibres, starch grains, mineral particles and very occasional pollen grains of species mostly occurring locally to the researcher's house including *Betula, Corylus, Fraxinus, Pinus*, Cruciferae, Compositae and Poaceae. The number of pollen grains of these species is listed in Table 2.

Insert Figure 3 here

Discussion

The unevenness of that decay in numbers of daffodil, tulip and false Christmas cactus through the first five WHO Protocols and the patchy recovery of their pollen subsequently can be ascribed to the variable effectiveness of the application of the protocols, despite the best efforts of the researcher to standardise procedures. It must be noted that this is a first study of this phenomenon. Further studies using larger numbers of subjects and a greater range of taxa would provide more solid evidence.

It is clear from these experiments that on average just under 1% of the initial pollen load on human hands is retained through at least one hand-wash episode using the WHO Protocol and that small numbers of pollen grains are retained for as many as 25 repetitions of the protocol, with some augmented by hand-scrubbing, and with other washing also occurring but not quantified. This is consistent with observations that pollen can survive machine washing (Bull et al. 2006; Zavada et al. 2007; Bryant and Bryant 2019) and dry cleaning (Mildenhall 2006a) of textile items. It seems that pollen adhesiveness and retention is slightly higher on the skin than on clothing as one thorough hand wash removes averagely 99.07% of pollen, whereas 99.9% is lost during one laundry cycle (Zavada et al. 2007).

These findings are credible because broadly consistent with results of studies of the retention of infectious bacteria and some viruses through hand-washing (e.g. Noskin et al. 1995; Kac et al. 2005; Liu et al. 2010) - which is why the WHO Clean Care is Safer Care Team (2009) recommend in the strongest terms the use of strongly bactericidal soap or an alcohol-based rub for routine hand-cleansing by healthcare professionals, with a more rigorous procedure for surgical staff.

These observations suggest that palynological investigation of human skin may be worthwhile in forensic contexts, even if some days and episodes of washing have elapsed after an individual may have come into contact with palynologically-distinctive flowering plants. This is especially the case, because abundant literature suggests that hand-washing was not always implemented rigorously in the recent past, even by medical staff, who might be expected to be highly motivated about hygiene than members of the general population (WHO Clean Care is Safer Care Team 2009: 66). It is likely that forensically-unaware individuals would have less rigorous washing habits than most medical staff.

It is possible, however, that fresh pollen retention may be greater than for other small particles because of the morphological complexity of the pollen exine and in particular because of the presence of the sticky, viscous pollenkitt and threadlike structures which may link zoophilous pollen grains (e.g. Hesse and Waha 1989: 151). The pollination mechanisms for most of the taxa in this study are predominantly entomophilous. The only exception is *Salix caprea*, which is technically ambophilous, in other words pollinated by both wind and insects, with the proportion being approximately 50:50 (Vroege and Stelleman 1990). They note that the pollen grains of *S. caprea* are rather sticky which is consistent with the partly-entomophilous pollination mechanism. The trends evident in Tables 2 and 3 and Fig. 3 further suggest that this difference in pollination mechanism is not significant in terms of pollen retention. Further work is necessary to investigate pollen retention on human skin for truly anemophilous taxa.

It seems from these results that the large grains of taxa such as daffodil, tulip and false Christmas cactus may survive more hand-washing episodes than the smaller grains of the other taxa studied. It also appears that slightly greater proportions of these larger grains (a mean of 1.43%) were retained through the first protocol, than were retained for the smaller grains (a mean of 0.71%). The reason for this differential survival is unknown. It seems counter-intuitive, since particles become more difficult to entrain in turbulent flows as they get smaller, once below ~60 μm (e.g. Dey and Ali 2019:

Fig 4). It may therefore be speculated that adhesion to their vectors by these large, heavy grains requires more effectively adhesive microstructures and pollenkitt than are required by taxa with smaller, lighter grains. On the other hand, the number of tests is very small and there is considerable variability in retention, so it is possible that these trends are no more than statistical noise. In practical terms, the observation of differential retention is likely to mean that assemblage composition is likely to change during hand-washing and therefore, that the forensic palynologist should rely on distinctive marker taxa in investigation of human skin.

It is extremely likely, given that pollen and bacteria survive hand-washing that other potentially forensically significant microscopic particulates may also be retained through hand-washing (e.g. microplastic, starch, phytoliths). This possibility should be investigated by relevant professionals.

Finally, the observation that pollen may be carried on human hands has some wider significance outside the possibility of its evidential use in forensic cases. First, carriage of pollen on the human skin means that this is potentially a way that contamination might be introduced into a crime scene or into forensic samples and this reinforces the necessity for rigorous protocols in crime scene investigation. Second, human skin is a pathway whereby contaminant pollen may be introduced into sampling for archaeological or palaeoecological purposes and investigators in these fields need to be aware of this possibility. In terms of microbiology, it is well known that viruses can be carried on the human skin. Experimental work (Liu et al. 2010) suggests that alcohol-based rubs are relatively ineffective against human norovirus, where hand-washing with soap and water is more effective but may still leave a viral load. There is now evidence that properly-formulated alcohol-based handrubs are effective against SARS-CoViD-19 and other non-enveloped viruses except when hands are very dirty, where hand-washing with soap and water may be more effective (Berardi et al. 2020). The difficulty of removing microscopic particulates by hand-washing, as demonstrated herein, makes essential the use of sufficient soap and a rigorous hand-washing procedure if this is the defence against the virus.

Conclusions

This project set out to investigate whether pollen on the human skin survived hand-washing regularly enough to make it a viable target for forensic palynological investigation. The WHO Protocol for hand-washing was used in an attempt to standardise the experimental procedure. The evidence from this study suggests that small numbers of pollen grains survive this rigorous hand washing protocol, with pollen of some taxa surviving several rounds of hand-cleansing, in one case as many as 25. It is therefore suggested that human skin can be a valid target for forensic palynological investigation, using a very simple methodology to extract and concentrate pollen for microscopic evaluation. Human skin may be a pathway through which contaminant pollen may reach crime scenes and archaeological excavations, and may contaminate samples.

This paper was written during the coronavirus pandemic of 2020. The importance of good hand hygiene, using sufficient soap and following rigorously the WHO hand-washing guidelines, cannot be stressed highly enough.

Acknowledgments

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References

Berardia, A., Perinelli, D.R., Merchant, H.A., Bisharat, L., Basheti, I.A., Bonacucina, G., Cespi, M., Palmieri, G.F. 2020. Hand sanitisers amid CoViD-19: A critical review of alcohol-based products on the market and formulation approaches to respond to increasing demand. International Journal of Pharmaceutics 584, 119431.

Bryant, V. M., Bryant, M. K. 2019. The role of palynology in forensic archaeology. In: Moran, K.S., Gold, C.L. (eds.) Forensic Archaeology: Multidisciplinary Perspectives. Springer, Cham. pp. 177-202.

Bull, P. A., Morgan, R. M., Sagovsky, A., Hughes, G. A. J. 2006. The transfer and persistence of trace particulates: Experimental studies using clothing fabrics. Science and Justice 46, 3, 185-195.

Clean Care is Safer Care Team. 2009. WHO guidelines on hand hygiene in health care. World Health Organisation. Geneva, WHO Press

Cwynar, L.C., Burden, E., McAndrews, J. H. 1979. An inexpensive sieving method for concentrating pollen and spores from fine-grained sediments. Canadian Journal of Earth Sciences 16, 5, 1115-1120.

Dey, S., Ali, S.K.Z. 2019. Bed sediment entrainment by streamflow: State of the science. Sedimentology 66, 1449–1485.

Hesse, M., Waha, M. 1989. A new look at the acetolysis method. Plant Systematics and Evolution 163, 3/4, 147-152.

Horrocks , M., Walsh, K. A. 1998. Forensic palynology: Assessing the value of the evidence. Review of Palaeobotany and Palynology 103, 1-2, 69-74.

Kac, G., Podglajen, I., Gueneret, M., Vaupré, S., Bissery, A., Meyer, G. 2005. Microbiological evaluation of two hand hygiene procedures achieved by healthcare workers during routine patient care: a randomized study. Journal of Hospital Infection 60, 1, 32-39.

Liu, P., Yuen, Y., Hsiao, H.-M., Jaykus, L.-A., Moe, C. 2010. Effectiveness of liquid soap and hand sanitizer against Norwalk Virus on contaminated hands. Applied and Environmental Microbiology 76, 2, 394-399.

Mildenhall, D.C. 2006a. *Hypericum* pollen determines the presence of burglars at the scene of a crime: An example of forensic palynology. Forensic Science International 163, 231–235.

Mildenhall, D.C. 2006b. An unusual appearance of a common pollen type indicates the scene of the crime. Forensic Science International 163, 236–240.

Mildenhall, D. C., Wiltshire, P. E., Bryant, V. M. 2006. Forensic palynology: Why do it and how it works. Forensic Science International 163, 163-172.

Montali, E., Mercuri, A.M., Trevisan Grandi, G., Accorsi, C.A. 2006. Towards a ‘‘crime pollen calendar’’—Pollen analysis on corpses throughout one year. Forensic Science International 163, 211–223.

Morgan, R.M., French, J.C., O'Donnell, L., Bull, P.A. 2010. The reincorporation and redistribution of trace geoforensic particulates on clothing: An introductory study. Science and Justice 50, 195–199.

Noskin, G.A., Stosor, V., Cooper, I., Peterson, L.R. 1995. Recovery of vancomycin-resistant Enterococci on fingertips and environmental surfaces. *Infection Control and Hospital Epidemiology* 16, 577–581.

Oeggl, K., Kofler, W., Schmidl, A., Dickson, J. H., Egarter-Vigl, E., Gaber, O. 2007. The reconstruction of the last itinerary of "Ötzi", the Neolithic Iceman, by pollen analyses from sequentially sampled gut extracts. Quaternary Science Reviews 26, 7-8, 853-861.

Piotrowska-Weryszko, K., Wydra, K., Korcz, N. 2017. The spectrum of sporomorphs collected from the surface of the skin and clothes of some people in Wlodawa County during the summer. Archiwum Medycyny Sądowej i Kryminologii/Archives of Forensic Medicine and Criminology, 67, 4, 241-253.

Vroege, P.W., Stelleman, P. 1990. Insect and wind pollination in Salix repens L. and Salix caprea L. Israel Journal of Botany 39, 125-132.

Webb, J. C., Brown, H. A., Toms, H., Goodenough, A. E. 2018. Differential retention of pollen grains on clothing and the effectiveness of laboratory retrieval methods in forensic settings. Forensic Science International, 288, 36-45.

Wiltshire, P. E. 2006. Hair as a source of forensic evidence in murder investigations. Forensic Science International, 163, 3, 241-248.

Wiltshire, P. E. 2009. Forensic ecology, botany, and palynology: Some aspects of their role in criminal investigation. In: Ritz, K., Dawson, L., Miller, D. (eds.) Criminal and environmental soil forensics. Springer, Dordrecht. pp. 129-149.

Wiltshire, P. E., Black, S. 2006. The cribriform approach to the retrieval of palynological evidence from the turbinates of murder victims. Forensic Science International, 163, 3, 224-230.

Wiltshire, P. E., Hawksworth, D. L., Webb, J. A., Edwards, K. J. 2015. Two sources and two kinds of trace evidence: Enhancing the links between clothing, footwear and crime scene. Forensic Science International, 254, 231-242.

World Health Organisation n.d. Clean Care is Safer Care. 2020.
https://www.who.int/gpsc/clean_hands_protection/en/

Zavada, M. S., McGraw, S. M., Miller, M. A. 2007. The role of clothing fabrics as passive pollen collectors in the north-eastern United States. Grana 46, 4, 285-291.

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364 Fig. 1. Deposit of daffodil pollen on researcher's hand. Arrow indicates location of pollen grains.

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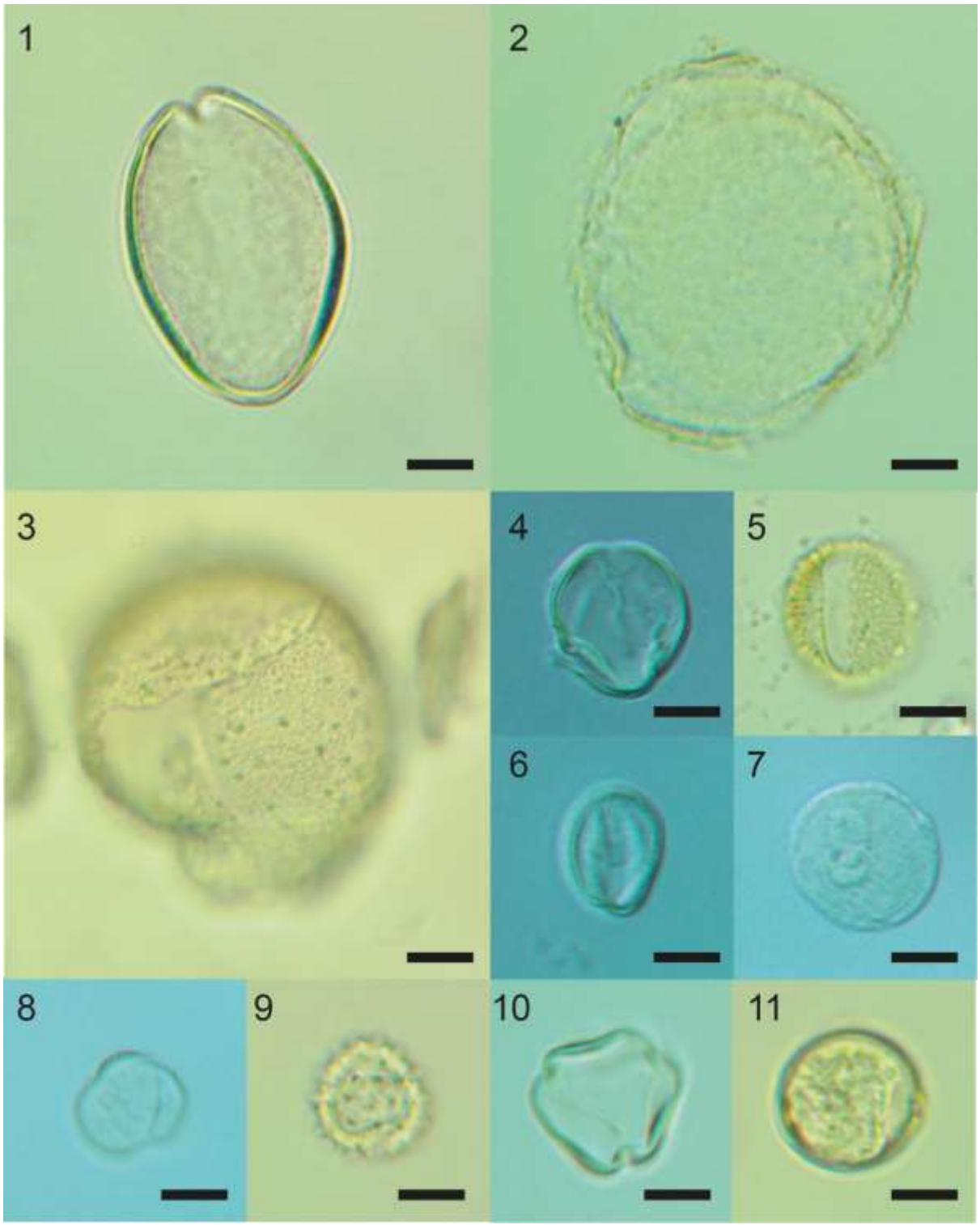
Figure 2. Plate showing typical specimens of the pollen grains recorded during this study. 1. *Narcissus pseudonarcissus* L., 2. *Tulipa xgesneriana* L. with cell contents, 3. *Schlumbergera truncata* (Haw.) Moran, damaged grain, 4. *Amelanchier alnifolia* (Nutt.) Nutt. ex M. Roem., 5. *Arabidopsis thaliana* (L.) Heynh., 6. *Anemone blanda* Schott & Kotschy, 7. *Caltha palustris* L., 8. *Salix caprea* L., 9. *Bellis perennis* L., 10. *Choisya ternata* Kunth, 11. *Betula pendula* Roth with cell contents. 1-3, 5, 9, 10 in transmitted light; 4, 6-8, 10 in Nomarski interference contrast. All scale bars are 10 µm.



375 Figure 3. Patterns of shedding of pollen grains through six sequential washes. No *C palustris* was
376 recovered during the 5th wash but one grain was recovered on the 6th wash.

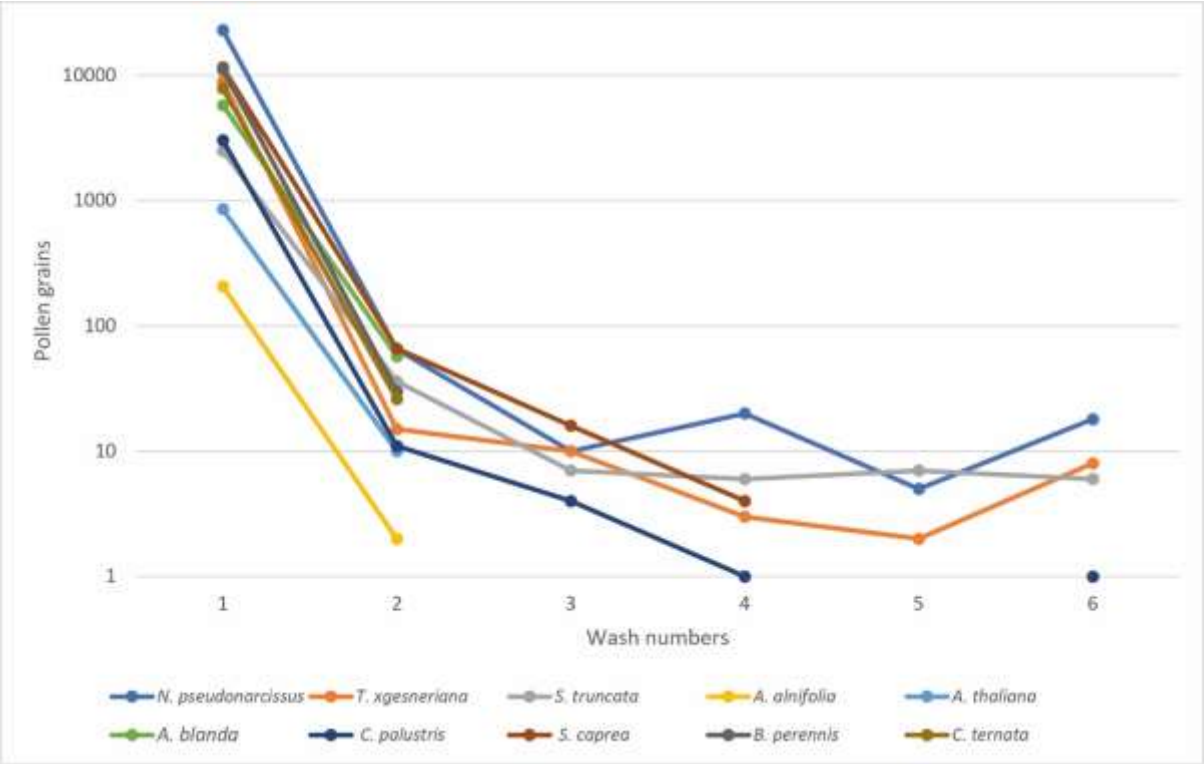


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Table 1. Characteristics of the pollen grains. Ten grains of each species were measured and dimensions for each axis are given as minimum(mean)maximum.

Species	Common name	Family	Morphology	Sculpture	Pollination mechanism	Dimensions (µm)	
						Polar axis	Equatorial axis
Narcissus pseudonarcissus L.	Daffodil	Liliaceae	Monocolpate	Microreticulate	Entomophilous	19(30.1)38	42{50.5)65
Tulipa x*gesneriana* L.	Tulip	Liliaceae	Monocolpate	Microreticulate-microechinate, perforate	Entomophilous	22(41.9)55	35(49.7)68
Schlumbergera truncata (Haw.) Moran	False Christmas cactus	Cactaceae	Pantocolpate	Microreticulate, microbaculate	Entomophilous	na	45(54.8)62
Amelanchier alnifolia (Nutt.) Nutt. ex M. Roem.	Juneberry	Rosaceae	Tricolporate	Very finely striate to psilate	Entomophilous	16(18.2)25	16(17.7)24
Arabidopsis thaliana (L.) Heynh.	Thale cress	Cruciferae	Tricolpate	Reticulate	Entomophilous	15(21.0)25	18{23.3)26
Anemone blanda Schott & Kotschy	Grecian windflower	Ranunculaceae	Tricolpate	Microechinate	Entomophilous	19(22.8)25	15(22.2)26
Caltha palustris L.	Marsh marigold	Ranunculaceae	Tricolpate	Microechinate	Entomophilous	12(20.0)22	17(20.8)24
Salix caprea L.	Goat willow	Saliciaceae	Tricolpate	Reticulate	Anemophilous and entomophilous	13(16.4)20	15(17.8)20
Bellis perennis L.	Daisy	Asteraceae	Tricolporate	Echinate	Entomophilous	15(20.6}25	18(19.9)25
Choisya ternata Kunth	Mexican orange	Rutaceae	Tricolporate	Microreticulate	Entomophilous	25(26.6)28	18(21.3)29

Table 2. Counts of pollen grains recovered using the WHO Protocol (plain text) and the WHO Protocol with scrubbing (**bold**). Pollen grains recovered in the initial WHO Protocol for the species are shown in *italics*.

Wash number	**Second study**			**Third study**							
	N. pseudonarcissus	*T.* x *gesneriana*	*S. truncata*	*A. alnifolia*	*A. thaliana*	*A. blanda*	*C. palustris*	*S. caprea*	*B. perennis*	*C. ternata*	Other pollen recorded
1	*22890*										
2	65										3
3	10										
4	20										
5	5										
6	**18**										
7	6	*8940*									
8		15									
9		10									2
10		3									
11		2									
12	**1**	**8**									
13			*2488*								3
14			36								9
15			7								7
16		1	6								2
17			7								2
18		**5**	**6**								**8**
19	2	1		*207*							92
20	**1**	**1**		**2**							6
21			1		*853*						26
22		**2**	**3**		**10**						1
23	3					*5764*					40
24	**2**	**4**				**57**					**20**
25	1	13					*3032*				7
26	**1**	**11**	**2**				**11**				**1**
27							4	*11548*			45
28			**2**				**1**	**66**			**1**
29								16	*11124*		42
30							**1**	**4**	**30**		**6**
31										*7850*	16
32										**26**	**1**

389 Table 3. Pollen retained through initial WHO protocol and recovered in later protocols

	N. pseudonarcissus	*T. x gesneriana*	*S. truncata*	*A. alnifolia*	*A. thaliana*	*Anenome*	*C. palustris*	*S. caprea*	*B. perennis*	*C. ternata*
Number recovered from initial WHO Protocol	22890	8940	2488	207	853	5764	3032	11548	11124	7850
Total recovered from subsequent protocols	161	76	70	2	10	57	17	86	30	26
Percentage retained through initial WHO Protocol	0.70	0.84	2.74	0.96	1.16	0.98	0.56	0.74	0.27	0.33

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