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Palynological Investigation of Mummified Human Remains

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Abstract

Pollen analysis was applied to a mummified homicide victim in Nebraska, U.S.A., to determine the location of death. A control sample showed the normal ambient pollen in the garage crime scene. Ambient windborne types, common in the air of the region, dominated the control. Internal samples were analyzed from the sacrum, intestine, and diaphragm. Microfossils were recovered from the rehydrated intestine lumen. The intestinal sample was dominated by *Brassica* (broccoli). The sacrum sample was high in dietary types but with a showing of ambient types. The pollen from the diaphragm was dominated by ambient pollen similar to the control samples. The discovery of diverse pollen spectra from within a single mummy was unexpected. They show that ingested and inhaled pollen mixed in the corpse. The data linked the decedent to a specific crime scene in her Nebraska home in the southern tier of eastern counties on the border with Kansas.

Keywords: forensic science, palynology, mummy, pollen, intestine, Nebraska, method

This note summarizes an analysis we conducted to determine whether or not the mummified victim of a homicide was killed in the region of her Nebraska home on the eastern Kansas–Nebraska border, or if she was killed elsewhere and transported as a mummy from some other locale. This was carried out based on pollen and botanical analysis. The cause of death was blunt force trauma as indicated by forensic pathology analysis. Several features of the case were relevant to pollen study. In the kitchen of the decedent's residence, investigators found the food consumed in the house before death which included broccoli, maize, and desert honey. The facts regarding the content of the kitchen were not shared with us prior to our analysis. Other relevant features came from the garage where the decedent was found. Importantly, the corpse was laid face down on an old section of carpet. This carpet served as a control sample for the ambient pollen in the immediate vicinity of the corpse. For this paper, "ambient" refers to wind-pollinated species (Table 1). A second palynologically relevant feature was the plastic envelopment of the victim's head. Investigation suggested that the plastic was wrapped around the head postmortem. This plastic would have protected the hair from the deposition of ambient pollen and therefore could be insightful as to the pollen in the air at or around the time the victim was killed. Other relevant features relate to the garage in which the decedent was found. In the garage was a van with New Mexico license plates which raised the potential that the mummy was transported from out-of-state. Another feature of the garage was significant for pollen analysis. The decedent and her partner had sheeted the windows of the garage, consequently limiting pollen transport from the outside environment into the garage. Therefore, the pollen in the victim's body would probably reflect inhalation and ingestion of ambient pollen and dietary pollen during the last few days before her murder. The pollen in her hair would represent the environment in which she lived before death. Geographically, the location of the victim's home is relevant. The home is in one of the southern tiers of east Nebraska counties that are agriculturally transitional from wheat production south of the Kansas–Nebraska border to maize production north of the border.

Because of the diversity of samples recovered from this case, we are addressing methodology applicable to recovering pollen and microfossils from intestinal contents and pollen from decomposition residue from within corpses. We are also presenting methods for pollen recovery from fabric and hair. Most importantly, we are presenting the method of pollen concentration by addition of pure, sterile *Lycopodium* spore tablets. Palynologists in geography, geology, and archeology have employed this method for decades as the basis for rigorous statistical analysis. In forensic science, some practitioners have resisted adopting this method. This leads to weaker presentations of data in legal settings. Importantly, the methods presented in this paper passed a Daubert hearing, and we believe this is the first case in U.S.A. legal history in which pollen data and methods were accepted into evidence. However, the data were not used in trial due to the suspect's admission of guilt in the pretrial stage. The original analysis was carried out in 2002 including investigative analysis of initial hair, sacrum, honey, and carpet samples. Overall, we had a short time frame to complete our preliminary reports to the State Patrol and County attorney. These analyses assisted in determining location and general season of death. We were granted permission to disseminate the results once the case was closed, and the initial product was a M.F.S. thesis (1). Subsequently, we reanalyzed the sediments from the analysis to increase pollen counts.

Table 1. Common names for pollen types and designation as either rare or common in the airborne pollen rain of eastern Nebraska.

Pollen Type	Common Names and Ambiguities*	Rare/Common
Ambrosia-type	Ambient ragweed and related genera	Very common
Brassica-type	Broccoli and related genera	Very rare
Cannabis/Celtis	Ambient marijuana or hackberry. The pollen from these plants is similar when degraded.	Common
Cheno am	Ambient pigweed and goosefoot species	Very common
Fabaceae	Bean species	Uncommon
Juniperus	Ambient cedar and juniper	Common
L.S. *Asteraceae*	Daisies	Somewhat common
Asteraceae type 2	Similar, but not identical to thistle	Uncommon
Cichorieae/Lactuceae	Dandelion and its relatives, also classified as Liguliflorae in older literature.	Uncommon
Morus	Ambient mulberry	Common
Pinus	Ambient pine	Common
Platyopuntia	Prickly pear	Rare
Poaceae	Ambient wild grass	Very common
Populus	Ambient cottonwood	Common
Quercus	Ambient oak	Common
Trifolium	Clover	Rare
Triticum	Wheat	Rare
Typha	Ambient cattail	Common
Ulmus	Ambient elm	Common
Zea	Maize	Rare

*Ambient refers to types that are general airborne types in eastern Nebraska.

Botanical analysis has long been an aspect of archaeological mummy studies, providing methods that can be applied in forensic settings (2–8). Dietary data from mummies comes from two sources: the analysis of solid food residue recovered from bowels (coprolites) and the pollen wash of rehydrated intestinal sections. Our knowledge of how pollen passes through the digestive tract is derived from experiments (9, 10). These experiments focused on long-term studies of ingestion/defecation patterns with known sources of pollen. The results showed that pollen, depending on pollen morphology, has variable peak passage through the intestine within a day or week after consumption. With regard to archaeological mummies, only 50% retain intestinal contents as fecal pellets (11:320). Indeed, in our laboratories' experience of 27 years of mummy analysis, approximately one-third of mummies retain coprolites. It is far more common to encounter fragments of desiccated bowel in the abdominal cavity, or powder in the abdomen and lower thorax. In our laboratory, we have had to develop methods for microfossil recovery from desiccated bowel sections, internal powder, as well as coprolites. Typically, pollen grains are not the only type of microscopic evidence found in such studies. Other microfossils include starch, plant cells, and animal hair (8). Macrofossils include seeds, fruit skin, nut fragments, and other large fragments of food.

We are presenting data from a homicide case in which the victim was mummified. We analyzed residual powder from the inside of the mummy. Additionally, we analyzed a small section of apparently empty intestine. Our methods of intestine wash were developed from archaeological analysis of mummies including an Iron Age bog mummy from the Netherlands (12), historic mummies from Lithuania (13–15), and recent analysis of intestinal sections from Italian Renaissance contexts (16). Reviews of palynology methods in forensic science do not address intestine analysis (17–19). Thus, we are presenting this method for the first time to the forensic science community.

Methods

The Palynology Laboratory in the School of Natural Resources at the University of Nebraska–Lincoln was built in 2005 specifically for forensic applications. As such, it is positive pressure facility with filtered air input. The filter eliminates particles smaller than 1.0 μm. For forensic work, all laboratory containers and tools are sterilized in 30% hydrogen peroxide to destroy any residual pollen that could contaminate the samples. All surfaces are processed with bleach. These procedures are necessary because the laboratory is used for pollen recovery from materials including honey, native bees, flowers and foliage, air samples, geological cores, and archaeological mummies. Therefore, exacting care is taken to prevent cross-contamination between these laboratory responsibilities and forensic cases. The facility is associated with the Forensic Science Program at the University of Nebraska, and a Forensic Palynology course is taught yearly in the laboratory. The laboratory also has a combination padlocking room for evidence storage.

The mummy analyzed was that of an adult female who was found in her Nebraska residence. That residence was in the town of Chester which is on the border of Nebraska and Kansas. Compared with several hundred mummies analyzed by us over 27 years, we were impressed with the nearly perfect state of mummification of the decedent. The near perfect state of preservation was based on our analysis consistent with recent methods (20). Well-preserved skin (with preservation to the follicle level) covered 100% of the body. Body hair, head hair, and fingernails were preserved in place. Breasts were preserved. Insect activity was limited to 37 dermestid-type burrows. Strong musculature retained its premortem form. Internal organ preservation was minimal. The diaphragm and shriveled lungs were evident. The abdominal organs were decomposed and desiccated. A short section of intestine preserved. Therefore, despite excellent external preservation, the internal organs were largely decomposed. In life, the decedent had residential connections in New Mexico and California. These areas would, in theory, be more conducive to mummification than Nebraska. Mummification through desiccation was unknown in the history of Nebraska crime and archaeology. Therefore, investigators entertained the possibility of cadaver transfer from southwestern United States to the Plains.

The decedent was a female who died suddenly from blunt force trauma to the cranium. She lay undiscovered for 6 years as revealed through Nebraska State Patrol investigations. When found, the body was unclothed but her head was covered in plastic closed with duct tape. Assuming that the plastic was placed around her head shortly after death, analysis of pollen from her hair might signal the environment in her immediate vicinity around the time she died. As plastic enveloped the head, it is reasonable to assume that contamination with pollen after death was limited. She lay prone, face down, on a loose section of carpet. The carpet was adherent to the front of the body. Dirt from the carpet was displaced to skin surfaces. We believed that pollen from the carpet would represent the accumulation of ambient pollen within the garage when she died.

Two external samples were taken as control samples to compare with the internal evidential pollen of the corpse: (i) 2.33 g sample of hair taken to represent the pollen in the air at and around the time of death and (ii) 21.55 g of carpet removed in the morgue taken to represent the pollen from a period of time before the homicide took place and probably represented the pollen accumulation over months and even years on the carpet.

Three internal evidentiary samples were taken immediately as the body was opened at autopsy. These included a 2-g sample from the area of the sacrum as defined by various archaeological papers (21–23). This sample was granular material which we suspected was organic residue of food. Previous archaeological work showed that the sacrum is an ideal area to test for dietary residue (21, 23). An intestinal fragment (14.0 cm × 1.5 cm) from the mummy was recovered and sealed in a paper bag. Later in the laboratory, the intestinal section was used to produce two subsamples for intestine interior and intestine exterior washes. It is important that quantification for this sample is presented in terms of pollen grains per mL of concentrated sediment recovered from a fluid wash of the intestinal tissue. Finally, a third sample of 11.4 g was recovered from the area inferior to the diaphragm in the upper abdomen. This was collected to test the diversity of pollen within the corpse.

Processing the Intestine

Oftentimes, mummy experts send to our laboratory sections of intestine without control samples. We have adopted the technique of washing the exterior of the intestine and maintaining the microfossils as a "control." Then, we open the section and wash the interior to evaluate dietary and medicinal residue. We call this method "intestinal wash." In this case, we rehydrated the entire section with 0.5% trisodium phosphate solution for 48 h. The exterior was rinsed and gently scraped with a sterile, plastic mini-spatula under a distilled water jet, and the fluid was collected in a 500-mL beaker. This sample beaker was labeled "exterior control." Then, the rehydrated section was opened longitudinally with a scalpel, and the interior was rinsed and gently scraped. The rinse fluid was collected in a 500-mL beaker and labeled "interior." Quantification of microfossils is carried out by adding a known number of exotic spores to a known volume or weight of sediment (24–28). For each of the interior and exterior samples, one tablet of *Lycopodium* spores (batch 124,961, each tablet containing 12,489 spores plus or minus 491, University of Lund, Sweden), was dissolved in 10% HCl within 50-mL beakers. These are tablets of pure spores. We dissolve spore tablet samples from each container of 500 tablets and analyze them for evidence of pollen contamination. So far, we have found no evidence of contamination in the 27 containers we have examined over the past 26 years. The 50-mL beakers were rinsed three times with distilled water to ensure that as many spores as possible were transferred to the samples. The samples in 500-mL beakers were then filtered through a 250-µm mesh screen. After a series of centrifugations, the total amount of residue was 2 mL from the intestine interior. Concentrating the external material was complicated by floating fatty material that adhered the microfossils in a mass. In previous cases, we determined that 4% KOH solution can dissolve fat-like material. We processed the exterior sample this way for a day (24 h). After that, the material was not dissolved. Therefore, we mounted samples of this fatty material directly on microscope slides for examination. In our experience with mummified intestinal sections, this complication of inert fatty material was unique to this case.

Processing the Sacral Sample and Loose Powder

The sacral sample was rehydrated using 0.5% trisodium phosphate solution as above. The sample, in a 500-mL beaker, was transferred to a stir plate. Using a standard magnetic stir bar, cleaned of all contamination, the sample was disaggregated so that microfossils were liberated. During this process, *Lycopodium* spores were added as described above. Then, the sample was screened through a 250-µm mesh, and microfossils in the recovered solution were concentrated by centrifugation. The powder sample from the area inferior to the diaphragm in the upper abdomen was processed in the same way.

Processing the Hair and Carpet

Wiltshire presented methods for processing hair for pollen (29). We modified this approach by replacing detergent as a major reagent with an alternative, pollen-free base. Hair and carpet samples were rehydrated using 0.5% trisodium phosphate solution. The samples were transferred in distilled water to 50-mL centrifuge tubes. The tubes were sonicated for 1 min and filtered through a 250-µm mesh screen and then centrifuged. One tablet of *Lycopodium* for each sample was dissolved in HCl as described above. The resulting material was less than 0.5 mL.

Preliminary Examination and Acetolysis

Before the sacrum and intestine samples were processed chemically for pollen retrieval, drops were transferred to microscope slides in glycerine and examined for cellulose plant residue and starch (30). The remainder of the samples residues were processed with standard acetolysis methods for pollen grain recovery. This procedure includes a 9:1 ratio of acetic anhydride to sulfuric acid (18, 31). Glacial acetic acid and water washes, along with centrifugation, followed the acetolysis procedure. Lastly, all the samples were washed with ethanol and transferred to vials with glycerine. Slide mounting and counting was then conducted. Final quantitative analysis was accomplished using standard pollen concentration calculation based on the following formula: $((m \div l) \times t)) \div x$.

m = microfossils counted;
l = *Lycopodium* spores counted;
t = number of *Lycopodium* spores added to the sample;
x = mass or volume of sample.

As recently reviewed (8), there has been a long literature dedicated to how many pollen grains researchers should count, beginning with Barkley (12). Today, it is standard archaeopalynology and geopalynology practice to obtain 200 grain counts at a minimum, based on extensive statistical analysis of diversity representation of pollen types in environmental samples (32). Among forensic palynologists, Horrocks (18) notes that most palynologists count at least 100 grains. Walsh and Horrocks (33) suggest that 100–200 grain counts are sufficient. The archaeopalynology/palynology focus on at least 200 grains is based on past research showing that 200 grain counts are reliable for the statistical representation of the pollen spectrum in typical samples (8, 22, 25, 26, 30, 32:394, 34, 35:666, 36, 37). However, for certain types of material such as honey and coprolites, it is advisable to count many more than 200 grains. For this case, we could not achieve a 200

grain count for all samples. Undoubtedly, this is due to the small samples we recovered from the corpse in the morgue. We overestimated the amount of pollen that could be present in small samples. For this reason, we analyzed our results statistically to determine whether differences in our data were significant.

The pollen counts were statistically analyzed using Minitab software (Minitab 16.2.4.4, Minitab Statistical Software, State College, PA). We analyzed the data via chi-square and ANOVA tests. We also applied a cluster analysis and a Friedman test of significance. We then compared the data graphically.

Ancillary Study of Honey

During analysis of the sacrum, a pollen grain from prickly pear (*Opuntia* or *Platyopuntia*) was observed. Prickly pear species are indigenous to a few Nebraska counties, but these are in the north central part of the state. Therefore, it is unlikely that this cactus pollen would come from the environment of the house. We processed a sample of prickly pear honey obtained from Arizona to assess the presence of pollen in honey. From this honey, 20 g was measured into a clean, glass beaker. Then, 20 mL of distilled H_2O and 200 mL of 95% EtOH were added to the beaker. The contents were mixed with a clean glass stirring rod and heated as needed to create a homogenous solution. Acetolysis was performed following a glacial acetic acid wash to remove excess water. An acetolysis solution of one part of H_2SO_4 to nine parts of acetic anhydride was added to the sample after the glacial acetic acid had been decanted from the centrifuge tube. The centrifuge tube was subsequently moved into a hot water bath for 3 min. The sample was centrifuged and decanted before being washed with glacial acetic acid. The sample was washed with distilled H_2O until the solution was clear. Slides were made by mixing 0.01 mL of each sample with a drop of glycerin onto a clean, glass microscope slide. Samples were topped with a 22 mm × 22 mm glass cover slip, sealed with a commercial nail lacquer, and examined at 400×.

Results

The common names for the identifiable pollen types are presented in Table 1. The pollen counts and derived pollen concentration values are presented in Tables 2 and 3, respectively.

The exterior sample of the intestine contained amounts of adherent fat-like paste. We analyzed 20 slides of this material and found no pollen. In contrast, the interior of the intestine was free of this substance, and recovery of microfossils was trouble-free. Therefore, the control sample, even though dominated by fatty material, served its purpose and showed that pollen was concentrated in the intestinal lumen and was not present on the outside of the external smooth muscle wall.

The following classes of microfossils were found in the pre-acetolysis wash of the intestine interior. Vascular cells were represented by tracheary elements (n = 116). Tracheary elements (tracheids and vessel elements) are elongated cells in the water conducting xylem of vascular plants. Plant epidermis was represented by only four sections of epidermis cells. Three maize starch grains, one altered by cooking, were found. It is noteworthy that 43 mites and 26 mite eggs were encountered. 114 *Lycopodium* spores were observed. Adapting the *Lycopodium* concentration method to these microfossils, there were approximately 6359 vascular cells per mL of intestinal sediment. For plant epidermis cell sections, 219 were present per mL, and 165 starch grains were present per mL. 2357 mites and 1425 mite eggs were present per mL of sediment.

Table 2. Pollen Counts from the samples.

	Intestine Section—Pollen	Sacrum Sample	Diaphragm Residue*	Carpet*	Hair
Lycopodium	1288	155	5	94	681
Ambrosia-type		3	11 (21%)	42 (69%)	29
Brassica-type	202	42	2		
Cannabis/Celtis	1		6	1	
Cheno am		9	18 (35%)	11 (18%)	8
Fabaceae		3			
Juniperus		1	2		
L.S. *Asteraceae*		3			
Asteraceae type 2				1	
Cichorieae/Luctaceae		2			
Morus			1		
Pinus		1			4
Platyopuntia		2			
Poaceae		4	9	3	4
Populus		1			
Quercus	1	4	3		1
Trifolium	1				
Triticum				1	3
Typha		1			
Ulmus					1
Unknown periporate		1			
Unknown exotics					4
Unidentifiable	1			2	
Zea mays		5		4	
Total count	**206**	**82**	**52**	**61**	**54**

*The difference between the diaphragm and carpet pollen spectra can be seen in the different percentages of *Ambrosia* type (ragweed and relatives) and Cheno am type (pigweed and goosefoot types).

Pollen was present in the intestine (Table 2). The pollen concentration calculations indicate that 1000 pollen grains per mL of intestine concentrate were present (Table 3). Pollen consistent with the genus *Brassica* was most common with 980 grains per mL of the final material recovered from the sample. Traces of oak, hackberry, and clover were present.

The sacrum sample contained clumps of maize starch. No other non-pollen microresidues were observed. Pollen was abundant in the sacrum sample. From this sample, over 3300 pollen grains per g were present. *Brassica*-type pollen was most common, and approximately 1693 pollen grains were present per g. Other types included maize (202 grains per g) and prickly pear cactus (81 grains per g). The prickly pear pollen was anomalous until we analyzed a sample of prickly pear honey. Prickly pear pollen was present in the honey. This suggests, but is not conclusive, that honey could be a source of ingested cactus pollen. There was a variety of environmental types including goosefoot/pigweed type, ragweed type, juniper, dandelion, grass, pine, cottonwood, oak, and cattail. All of these types are typical of the eastern Nebraska/Kansas border. The maize pollen was probably environmental as well, although it is conceivable that this was ingested with a food source. Pine pollen carries on the wind for very long distances, and this may have originated with pines growing far away from the crime scene.

The pollen counts from the diaphragm evidentiary sample show a reduced representation of dietary pollen. From this sample, there were 11,504 pollen grains per g of sample. Environmental pollen is abundant and diverse. Ragweed pollen composed 21% of the count. Pigweed/goosefoot type composed 35% of the count. The other environmental types composed 40%

Table 3. The pollen concentration data derived from the pollen counts.

	Intestine Section—Pollen/mL*	Sacrum Sample/g	Diaphragm Residue/g	Carpet/g	Hair/g
Ambrosia-type		121	2412	259	228
Brassica-type	980	1693	239		
Cannabis/Celtis	5		1316	6	
Cheno am		363	3948	68	63
Fabaceae		121			
Juniperus		40	239		
L.S. *Asteraceae*		121			
Aster type 2				6	
Luctaceae		81			
Morus			219		
Pinus		40			32
Platyopuntia		81			
Poaceae		161	1974	19	32
Populus		40			
Quercus	5	161	658		8
Trifolium	5				
Triticum				6	24
Typha		40			
Ulmus					8
Unknown periporate		40			
Unknown exotics					32
Unidentifiable	5			12	
Zea mays		202			
Total concent	1000	3305	11,005	376	427

*It is important to note that quantification of the intestine sample is presented in terms of pollen grains per mL of concentrated sediment recovered from a fluid wash of the intestinal tissue. The other samples were weighed samples

of the results. This spectrum contrasts sharply with the samples from the abdomen. The control sample taken from the carpet was dominated by ragweed and pigweed/goosefoot types, and a few other wind-born environmental types.

The hair sample produced 54 pollen grains. Fifty were common environmental types such as ragweed and pigweed/goosefoot types. Four pollen grains were exotic and are not represented in Nebraska pollen rain. We could not identify these types, and they appear to be from insect-pollinated tropical plants.

Statistically, the Friedman test showed that the hair sample was not comparable to the others. ANOVA demonstrated there was statistical difference between the intestine, sacrum, diaphragm, and carpet samples. To compare sacrum and intestine samples, we employed a Mann–Whitney test. This test revealed no statistical difference between sacrum and intestine. The intestinal wash is dominated, almost exclusively, by *Brassica*-type pollen. The sediment from the sacral area is divided almost equally between *Brassica*-type pollen and environmental types. The diaphragm sediments are mostly environmental with a very small presence of *Brassica*-type pollen. The carpet is exclusively environmental. The intestine interior is almost exclusively dietary.

Discussion

It is clear that the internal intestine pollen counts are different from the diaphragm counts. The abdomen was not open prior to the autopsy. The presence of ambient pollen in the abdominal powder suggests that environmental pollen entered the mummy at some time. The sacrum counts are influenced by both of these areas. This indicates a mixing of pollen within the corpse. The main contrast is between the intestine and diaphragm. The former is dominated by *Brassica*-type pollen and the latter by ambient types. Ambient environment types consist of windborne pollen grains that are well represented in the air. Clearly, the consumption of *Brassica*-type flowers, probably in the form of broccoli or related plants (which are all varieties of *Brassica oleracea*), was the source of the intestinal pollen. We can think of two reasons for the presence of environmental pollen in the diaphragm. This sample could have been affected by airborne pollen released from the decomposing lungs. Therefore, pollen breathed in by the decedent might be represented in the diaphragm sample. Alternatively, this pollen spectrum could be influenced by the pollen spectrum from the carpet on which the decedent lay, face down. Fragments of the carpet adhered to her skin. The contamination of the internal pollen with carpet pollen could have occurred at the time of autopsy. We believe that the vibration of the autopsy Stryker saw could have caused fragments of carpet to fall into the body, thereby adding contamination to the internal pollen spectrum.

To resolve this issue, we looked at the predominance of ragweed versus goosefoot/pigweed type. From the carpet, 69% of the pollen is from ragweed and 18% is from goosefoot/pigweed type. This contrasts with the diaphragm sample in which 21% of the pollen is from ragweed and 35% is from goosefoot/pigweed type. This is a noteworthy contrast between the dominant types of the two samples. This difference suggests that the carpet sample did not influence the diaphragm sample to a great degree. Therefore, we tend to think that the airborne pollen in the diaphragm originated with inhaled pollen in the lungs.

The question as to where the homicide took place is resolved by the pollen data. From the large-scale perspective, the pollen types are completely consistent with the pollen of the Central Plains of the United States. On a smaller scale, the pollen spectrum of the southernmost tier of Nebraska counties on the Kansas border is evident. The presence of small amounts of maize and wheat pollen in the decedent and at the crime scene is important. Wheat pollen was found in the decedent's hair and on the carpet. Maize pollen was found on the carpet and in the sacrum. The dominant agricultural product of Kansas is wheat. Across the border in Nebraska, maize is the main agricultural product. In eastern Nebraska, the southernmost counties are planted with wheat and maize. Indeed, the Nebraska–Kansas border and these counties mark the northern boundary of eastern Nebraska wheat production. The presence of both cultigens is very strong evidence that the homicide took place at the decedent's home on the border between the states of Nebraska and Kansas. The fact that wheat pollen occurred in the hair of the suspect indicates that she was in this agricultural zone at or around the time of death. It should be noted that because of the possible criminal activities of the decedent and her partner, their garage was lined with layers of sheeting to keep the contents away from the prying eyes of neighbors. Air movement in the garage was deadened where the body was deposited, and this limited pollen entrance into the space. All in all, final investigation concluded the deceased was killed on the property. There is no evidence of transport of the victim from southwestern states.

On the finest scale, the find of dietary pollen that could be linked to the inventory of the decedent's kitchen links the homicide to the house where the victim was found. We found maize starch and *Brassica*-type pollen in the internal evidentiary samples. The last foods eaten in the house were corn and broccoli. Prickly pear pollen was present in the sacrum. The prickly pear pollen was found in our test of desert honey. Desert honey was found

in an open container in the kitchen. Therefore, it is possible that the cactus pollen reflects desert honey. Thus, there are palynological and botanical matches between the foods being consumed in the kitchen and the foods in the internal evidentiary samples. It is highly likely that the decedent was killed in her home.

To achieve the minimal 200 grain counts, we suggest taking large samples of internal residue. In this case, the analysis of the carpet control sample, external to the mummy, defined the types of ambient pollen present in the crime scene environment. The hair contained insufficient pollen for a representation of the environment. Pollen exotic to Nebraska suggests that the victim perhaps used a specific product on her hair that carried pollen from a plant source external to the Great Plains.

For forensic science, we are presenting a new approach to collecting and quantifying pollen and microbotanical data. We demonstrate that the intestinal wash is a good method of recovering dietary residue. We also show that pollen mixing can occur within a corpse. We did not expect to find significant differences in pollen counts from within the mummy. The fact that distinct pollen spectra occurred within the decedent suggests that inhaled pollen and ingested dietary pollen mix during the decomposition of internal organs. We intend to test this suggestion with other mummy samples in our collections. We believe that until this analysis, the possibility that lung decomposition releases substantial amounts of pollen into the corpse has been unrecognized.

It is appropriate to address the resistance of some forensic pollen analysts to the use of quantification that can be offered by the *Lycopodium* spore tablet technique. In forensic science, there is a regional affinity with this method. Among US forensic palynologists, pollen concentration methods are used (17). However, there is a surprising resistance to calculating pollen concentration among some forensic palynologists. The resistance to pollen concentration quantification is exemplified by the following quotes from forensic science literature. "Exotic marker spores were not added due to pollen and spore concentration values having little worth in such situations" (38:206). This reference is to a study conducted on the secondary deposition of bodies from seven mass graves comparing the pollen spectra to seven primary massacre sites. The goal was to determine to which secondary sites were bodies moved from the primary sites. The author was partially successful using general pollen profiles represented by percentages of general pollen categories. We believe that if he had use pollen concentration to determine absolute pollen quantities in his samples, the study would have been more successful as he would have been able to determine the diminishing numbers of primary grave pollen types as these were transported to, and mixed with, secondary site sediments. In another article, the authors stated "No attempt is made to assess the concentration of palynomorphs by the standard method of counting in relation to known numbers of added exotics. This technique is inappropriate where counts cannot be related to absolute amounts of original sample" (39:226). This study focused on the pollen spectra of surface soil and grave sediments in an attempt to determine whether the pollen in a homicide victim's nasal aperture was derived from surface or subsoil pollen. This would have been an ideal opportunity to apply pollen concentration as the amount of pollen would be the key to answering the question. The difference in pollen concentration between the surface and the grave fill, compared to the amount of pollen in the nasal sediment, would have resolved this issue. Without quantification, the authors' assertion that "… the soil in the turbinates was highly polleniferous, …. This was not the case for the grave soil" can not be backed up with pollen concentration values (230:39). The authors are in error when they state that samples "cannot be related to absolute amounts of original sample." We have found that the *Lycopodium* method of quantification can be applied to any source. In analysis of cloth, we can quantify in terms of pollen grains per cm^2. For intestinal fluids, honey, mucous, and other liquid sources, quantification can be determined in terms of pollen grains per mL. For solids including tobacco (4), sediments, soil from turbinates, quantification can be carried out in terms of pollen grains per g. In a third paper, an author asserted "No attempt is made to assess the concentration of palynomorphs by the standard method of counting in relation to known numbers of added exotics as this technique is inappropriate for forensic investigation" (29:242). This is a blanket statement that did not define propriety or impropriety of quantification. Why would quantification and statistical analysis be inappropriate for forensic science? From our perspective, rigorous quantification is appropriate in every scientific application of palynology. It is noteworthy that the data and the method from our analysis of the Chester mummy were judged to be admissible in court proceedings. This demonstrates that *Lycopodium* pollen concentration is entirely appropriate in forensic science applications.

For established palynologists in geology, archaeology, and geography, *Lycopodium* quantification has been the standard method for decades (40). The formal adoption of this method by the American Quaternary Association signals the trust palynologists place on the quality of these pure *Lycopodium* spore tablets (8). Spore tablets for calibration of pollen analyses were first produced and distributed by Dr. Jens Stockmarr, Copenhagen. In October 1980, this service was taken over by the Department of Quaternary Geology in Lund. It is performed as an official commission approved by the University of Lund. *Lycopodium* spores are collected and then processed in sterile laboratory conditions that prevent contamination with pollen. We suggest that it is time that forensic palynologists employ this long-trusted method.

The value of this method relates to the forensic questions one might be asked. What did the victim eat? Did the homicide occur *in situ*? We have addressed these questions in this case study. When compared to simple counts and percentages sometimes presented in the forensic pollen literature, pollen concentration data are amenable to statistical tests and the determination of absolute magnitude of pollen saturation of a study site. With regard to pollen ingestion, the intestinal tract concentrates pollen in very high loads that can run into the millions, especially for people who ingest polliniferous foods such as honey, flowers such as broccoli or asparagus, bee pollen, etc. For statistical comparison, a number of tests can be applied to these absolute numbers for tests of significance. In sediments, there is a great value as well. For example, in recent cases of testing soil surrounding a corpse, we found that the pollen concentration data signaled that pollen from the corpse filtered into the underlying sediments. This was indicated by decreased pollen concentration values with increased percolation distance from the corpse. Only through pollen concentration analysis can these perspectives be addressed.

Historically, this case is of importance. We believe that this is the first case to be subject to a Daubert hearing. The legal staff of both sides read a portion of the extensive peer-reviewed literature on this pollen concentration method. They approved the method and the data presented in this paper as appropriate for legal proceedings.

A final question is, why did the decedent mummify so well in Nebraska? Uniquely, climate and drought data showed that her mummification could have been attributed to an early 2000's drought that her region experienced. Data for 2003–2006 show that the area, including Chester, Nebraska, experienced drought conditions ranging from mild to severe (41). From that perspective, the decedent's mummy was unique in the field of mummy studies. Death occurred in the early winter months, which is the most arid period for the Central Plains region. It is possible that the drought, combined with seasonal aridity, resulted in the thorough mummification of this individual.

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