Forensic Palynological Investigation of Marijuana Use: From Paraphernalia to Corpses
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ABSTRACT
In 2010 we analyzed pollen samples from a human skull and a variety of objects. We found that Cannabis sativa pollen transfers readily from its source and contaminates surfaces and objects in the environment where it is used. Non-cannabis pollen on pipes and trash materials shows locally where marijuana was prepared for smoking and where it was smoked within Lincoln, Nebraska. Importantly, the discovery of Cannabis in the nasal aperture of a corpse showed that the decedent was in the vicinity of marijuana at the time of death. The marijuana pollen, combined with other pollen types, shows that the decedent did not die where the body was found.

METHODS
There are various procedures for recovering pollen from physical evidence for microscopic examination ranging from fast and simple to laborious and dangerous. Which method is used depends on the nature of the source material. Recovering pollen from clothing, boxes, bags or other items that came into contact with cannabis is relatively easy and involves vacuuming and acetylation. Acetylation involves treatment of samples in a mixture of sulfuric acid and acetic anhydride. The cellulose filter from the vacuum is dissolved in acetic acid. Extracting pollen from cannabis samples is slightly more complicated. A solution of potassium hydroxide is used to dissolve the resin that holds pollen to foliage. Then the sample is sonicated to release the pollen into solution. Next, the pollen is screened through a 150-micrometer mesh and the pollen and other microscopic remains are processed through an acetylation solution. Cannabis pipes must be soaked in potassium hydroxide solution and then the resin with ash and pollen can be freed from the pipe. The microscopic residue is then processed with acetylation solution. The most complex process is required of soil samples. Soil samples must be processed through a chemical series involving bals in hydrochloric acid, acetylation solution, and hydrofluoric acid.

Pollen was mounted on microscope slides in glycerin. A cover slip was then placed on the pollen preparation sealed with commercial nail polish. A minimum of 200 pollen grains were counted and identified. Pollen counts were made with a Jeneval compound microscope using 40x and 100x objectives with 10x oculars at DIC and bright field settings. A Zeiss microscope was used to do the final imaging. For each pollen type, the quantity was calculated using this formula:

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\text{Pollen Concentration} = \frac{(p/m)e}{x}
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where:
- p: pollen grains counted
- m: marker grains counted
- e: number of exotic marker pollen grains added
- x: weight, area or volume of evidence

WHAT CAN BE LEARNED: TRACING CANNABIS ASSOCIATION
Processing cloth, plastic or cardboard evidence can show whether or not cannabis has been stored in that evidence. Cannabis produces large numbers of pollen grains per plant. This pollen readily transfers to objects that come into contact with source material. In this study, cannabis pollen was found in 3 baggies, a mouse pad, styrofoam and a box. The amount of pollen was very small in the baggies, amounting to less than 10 pollen grains. Pollen was more abundant from the box and styrofoam, as well as stems and seeds. However, pollen was most abundant on a mouse pad that was used to sort cannabis. 11 marijuana grains per inch of the mouse pad were recovered. In addition to verifying that these items came in contact with cannabis, the mouse pad also revealed pollen of walnut, elm, and oak which grew in the immediate area where the pad was used. Other pollen from greasewood, buffalo berry and spruce are from the western region of North American and probably represent the cultivation region of the cannabis sorted on the mouse pad. Closest to Lincoln, these three plants grow in the Black Hills of South Dakota. But they can also be found in mountainous areas of the western states.

WHAT CAN BE LEARNED: GEOGRAPHY OF CULTIVATION
Pollen from cannabis samples themselves can show where it was grown. Unlike the mouse pad which may have pollen from cannabis grown in several geographic areas, pollen from bulk samples of cannabis can be assumed to represent a single geographic origin. An especially resinous sample contained a diversity of pollen types including mesquite, wormwood, creosote, grass, oak, pine, sunflower, cattail and alder. These plants grow in the lower Sonoran Desert of Mexico and Arizona. The alder and cattail show an association with a permanent waterway. This cannabis was grown near a stream or river in a lowland desert. The Sonoran Plains of Sonora Mexico is the most likely cultivation source for this sample. The absence of olive and mulberry, which are introduced plants in desert cities, rules out urban areas.

WHAT CAN BE LEARNED: WHERE PIPES WERE SMOKED
Pollen collects in pipes from the cannabis itself and also from the surrounding air as the user inhales. If the user is smoking in an area with unique plants, the pollen in the pipe signals that plant community. We analyzed 2 pipes. The pollen was dominated by cattail and non-native conifers common in Lincoln parks. This association of plants can be found along the streams in two of Lincoln’s parks within walking distance of one of Lincoln’s high schools. It is very likely that the pipes were used at these locations.

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GEOGRAPHY, CONTINUED
The pollen in the cannabis samples is typical of the Sonoran Desert. Determining which elevation in the desert is based on looking at the distribution of the pollen types compared to modern samples. The University of Arizona is a source for such data. Looking at the U of A plot of pollen types, all types are only found in the lower Sonoran Desert.

POLLEN AND BONES
Pollen recovered from the nasal aperture of skulls can be used to determine season and location of death. In an unresolved case, and for which analysis is still underway, pollen from the nasal aperture shows that the decedent’s last breaths were not taken where the body was found. The body was found in mixed wood and field area. Pollen from the crime scene was poorly preserved and represented primarily ragweed, pigweed, grass and other herbaceous plants. Pollen was recovered from the nasal aperture of the decedent’s skull. This pollen was well preserved. Corn and cannabis pollen were identified in this sample, indicating that the decedent was in a mixed field of corn and cannabis at or around the time of death.