2015

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Arguelles, Paulette; Reinhard, Karl; and Shin, Dong Hoon, "Forensic Palynological Analysis of Intestinal Contents of a Korean Mummy" (2015). *Karl Reinhard Papers/Publications*. 72.
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Forensic Palynological Analysis of Intestinal Contents of a Korean Mummy

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Abstract

Experimental studies show that pollen resides in the intestinal tract for a minimum of seven days to at least 21 days. Because of this long residence time, pollen analysis is an important avenue of forensic research. Pollen provides evidence of the environment of the decedent as well as foods and medicine. We analyzed a coprolite recovered from a Korean mummy. The decedent was a high-ranking general who lived during the 16th or 17th centuries. Twenty pollen types were recovered. These ranged from 100s to 10,000s of pollen grains per gram of coprolite. Importantly, comparison of the coprolite pollen spectrum to modern aeropalynology studies of Korea suggests that the general died in winter between middle November to late February. Economic pollen types were most abundant. Economic refers to dietary, medicinal, spice, and beverage types. Dietary pollen types include pollen from *Oryza* (rice), *Eriogonum* (buckwheat), Brassicaceae (mustard family), and Solanaceae (tomato-chile pepper family). Pollen consistent with dandelion is present and may represent its use as food. Tens of thousands of grains from water plants, bur-reed or cattail, dominate the pollen spectrum. We believe that this was introduced with water. The large numbers of water-related pollen suggest that the general consumed broth, tea, or soup for a considerable time before death.

Keywords: mummy, mummies, mummified

Introduction

A Korean mummy of the Joseon Dynasty (1392–1910) was discovered in Gangneung City located on the eastern coast of the Korean peninsula (Lee et al., 2009). The individual was an elderly man and a high-ranking general who had lived during the 16th and 17th century in Korea. This study focuses on analysis of a coprolite found in the intestinal tract (Fig. 1). We demonstrate that even after hundreds of years of burial, palynological analysis can still be done in the aim of determining the types of food eaten and the general season in which the general died. Previously, information was published about the general's

mummified corpse, such as CT images of well-preserved intestinal contents (coprolites) in the pelvic cavity (Lee et al. 2009).

Materials and Methods

A small coprolite sample (0.67 g) was placed in a 50 mL centrifuge tube and rehydrated with 0.5% trisodium phosphate for a 48 hr period. A dissolved tablet of 12,540 (batch # 124961) *Lycopodium* spores were added to the sample to allow for determination of each type of pollen following the methods of Maher (1981) and Reinhard et al. (2006). The sample was disaggregated in distilled

Fig. 1. Ct-scans images from Lee et al., (2009). Asterisks indicate feces within the pelvic girdle.

water in a 600 mL beaker using a stir bar and a stir plate, followed by a screening through a 250 μ m mesh with distilled water. The water and microscopic residues that passed through the screen were collected and centrifuged. Microscopic remains were examined for starch and phytoliths.

An acetolysis procedure was used to dissolve potential cellulose, hemicellulose, and chitin (Reinhard et al., 2006). The sample was washed with glacial acetic acid, mixing the acid with the sediment. After the acid was decanted, acetolysis solution (8 pts acetic anhydride to 1 pt sulfuric acid) was mixed into the sediment. The microscopic residue was heated at 99°C for 10 min, after which the solution was disposed. After one glacial acetic acid wash, the sample was then washed repeatedly in distilled water until the supernatant was clear.

The sediment sample was mounted on microscope slides with an additional drop of glycerin. A cover slip was then placed on the preparation and sealed with commercial nail polish. Following palynological procedures, a minimum of 200 pollen grains was counted and identified. Pollen counts were made with the Jeneval compound microscope at 403 and 1003 objective with 10x oculars using DIC and bright field settings. A Ziess axiomat compound microscope was also used for analysis. Imaging was done with the Jeneval system. For each type of pollen, the

concentration was calculated per gram of sediment. This was calculated by:

$$\text{Pollen Concentration} = [(p/m)e]/w$$

1. p : pollen grains counted
2. m : *Lycopodium* marker grains counted
3. e : number of *Lycopodium* marker pollen grains added
4. w : weight or volume of sediment

Results

There was a variety of pollen types recovered which represents the general's diet and environment (Table 1). The calculated pollen concentrations ranged from hundreds to tens of thousands of pollen grains per gram of coprolite. Traces of Apiaceae (carrot family), Caryophyllaceae (carnation family) were found in preliminary scans for starch and phytoliths. The pollen can be divided between environmental types that were fortuitously consumed and economic types that were consumed with food or beverage.

Environmental Pollen

The most common pollen type is *Typha-Sparganium*, cattail or bur-reed. Both cattail and bur-weed are aquatic plants that produce identical pollen. The pollen of *Typha*

Table 1. Pollen counts from the coprolite with calculate pollen concentration values in terms of pollen grains per gram

	138	Pollen grains per gram
Lycopodium		
Economic types (from foods, beverages, or medicines)		
Asteraceae, <i>Achillea</i> , or <i>Chrysanthemum</i> type	19	2,569
Asteraceae, <i>Achillea</i> , or <i>Chrysanthemum</i> type broken	3	406
Asteraceae, Cichorieae (Lactuceae)	11	1,487
Brassicaceae	3	406
Brassicaceae broken	2	270
Fabaceae	42	5,678
<i>Fagopyrum</i>	4	541
Lamiaceae, <i>Salvia</i> -type	8	1,082
Liliaceae, reticulate	1	135
<i>Oryza</i>	6	811
<i>Oryza</i> , shredded	1	135
Ranunculaceae periporate	1	135
<i>Rhus</i> -type	1	135
Solanaceae	2	270
Solanaceae clump (2)	1	135
Environmental types (Pollen inhaled, drunk, or fortuitously ingested)		
<i>Celtis</i>	3	406
<i>Fraxinus</i>	1	135
<i>Pinus</i>	7	946
Poaceae wild	1	135
<i>Quercus</i>	1	135
<i>Salix</i>	1	135
<i>Typha-Sparganium</i>	75	10,140
Other		
Fern	1	135
Unidentifiable	2	270
Unknown Euporbiaceae type	1	135
Unknown verrucate tricolpate	6	811
Total	204	27,578

is classified into two types, *T. latifolia* and *T. angustifolia*. *T. latifolia* pollen grains are released in clusters of four called tetrads. *T. angustifolia* type occurs in single grains called monads. Bur-weed is released in monads. There are two possible reasons for the presence of this pollen in the mummy. Cattail pollen was, and is, collected in some parts of the world as food (Reinhard et al., 2006). Alternatively, because cattail and bur-weed are water plants, their pollen can also be drunk with water. When cattail is eaten, the numbers of pollen grains per gram spike from 100,000s per gram of coprolite to 1,000,000s of grains (Reinhard et al., 2006). In this mummy, 10,140 pollen grains per gram were found. This is a relatively low value and probably represents water consumption.

The other environmental types include *Fraxinus* (ash), *Pinus* (pine), *Salix* (willow), *Celtis* (hackberry), and *Quercus* (oak). We found seven pine grains, three hackberry grains, and one grain each for the other types. This is a very low representation of environmental pollen. Normally, environmental pollen constitutes 94% of the pollen count (Hevly, 1989; Bryant and Holloway, 1984). The 43% representation of environmental pollen for this mummy is noteworthy, especially considering that just 7% of the pollen comes from wind-pollinated trees.

Economic Types

The economic types consist of insect dispersed pollen. Normally, insect dispersed pollen makes up 2–4% of the normal pollen rain. In this case, it makes up more than half of the pollen. Insect pollinated types are not as well

described as wind pollinated plants. Therefore, it can be very difficult to identify pollen of insect pollinated plants to genus and species level.

The most abundant economic pollen type appears to be in the Fabaceae, bean family (Fig. 2). The pollen concentration value for this type is 5,678 grains per gram. Bean family pollen is commonly eaten with honey. The flowers of some genera are edible, including species of *Pisum* (pea), *Phaseolus* (bean), and *Trifolium* (clover). The Fabaceae pollen in this sample is smaller than any of these, and is most consistent with *Melilotus*, sweet clover, but is 5 μ m smaller than sweet clover. Its botanical origin is still unknown.

Another common pollen type is a spiny (echinate) type that is consistent with *Achillea*, yarrow, or *Chrysanthemum* (Fig. 3). Both are insect-pollinated and therefore should not be present in the air. The pollen is double walled (tectate) with the walls separated by minute pillars (columellae). Akyalçin et al. (2011) and Meo and Khan (2008) present detailed morphological description of yarrow and chrysanthemum, respectively. Their documentation shows that yarrow species have pollen that is microporate whereas chrysanthemum pollen does not. The pollen from the mummy is not microporate and therefore appears to be more consistent with chrysanthemum. The high numbers of this pollen type, 2,975 pollen grains per gram, indicates that it was intentionally consumed. Many of the 85 yarrow species are medicinal. Of the 30 species of chrysanthemum, many are used as medicines and beverages. Of the beverages, chrysanthemum tea and wine are

Fig. 2. Bean family pollen grains in equatorial view. There was an abundance of this type of pollen in the sample. Three pollen grains are shown in this series of images. The walls are smooth or microreticulate. Each pollen grain has three grooves from polar area to polar area. Each groove has a pore making these grains trocolporate. The pores range from being large and round as shown in the middle and lower series of picture to small and oval as shown in the upper picture.

Fig. 3. An *Achillea* (yarrow) or *Chrysanthemum* pollen grain in polar (upper row) and equatorial (lower row) views. The upper left image clearly shows that there are two walls separated by a series of collumellae. This is a tectate grain. The grains are ornamented with spines (echinate) and spines are wide at the bases. The tricolporate morphology is shown in these images and the pore is very distinct as seen in the equatorial views.

made in Korea (Kim et al., 2008). The pollen of this type is deformed and torn. The deformation of the pollen suggested that it was prepared in a way that would weaken the pollen walls.

Cichorieae (sometimes Lactuceae) refers to an insect-pollinated subgroup of the sunflower family (Fig. 4). The Lactuceae is widely distributed around the globe. It includes mostly herbs among its 1,500 species. Several species are used as medicine, food, and beverage. Common economic plants include lettuce, chicory, and dandelion. The presence of 1,487 Cichorieae pollen grains per gram of coprolite shows that flowers, or something made from flowers, were consumed. Dandelion (*Taraxacum* species) was used in flavoring rice wine in Korea (Kim et al., 2004). Salads made of dandelion buds and leaves were eaten in Asia as well.

The Lamiaceae, mint family, is also represented by large numbers of pollen grains per gram (Fig. 5). The mint family includes two types of pollen; tricolporate with three grooves around the equator and stephanocolate which has six grooves. The pollen in the mummy is stephanocolate. Its specific morphology is consistent with the genus *Salvia*, sage, in the University of Nebraska pollen reference collection and as described by Orsini et al., (2006). However, a

sage with morphologically similar pollen is listed as fundamental Chinese medicinal plant and for Korea (Moon et al., 2008). This is *Agastache rugosa*, which is used for abdominal pain (Wu and Raven, 1994:106).

Rice (*Oryza sativa*) pollen grains were found (Fig. 6). Compared to grass and cultivated rice grains are distinct. Both are spheroidal, monoporate, annulate grains. However, rice grains are larger in overall diameter. The diameter of the annulus around the pore area also larger in rice. Clusters of rice grains were found which shows that rice, with adherent pollen grains, was consumed. The absence of rice starch indicates that the rice was eaten in a cooked form. The cooking gelatinized the starch but did not alter the pollen.

Brassicaceae (mustard family) pollen was represented by over 900 pollen grains per gram of intestinal content. The pollen is consistent with the genus *Brassica*, which includes the cruciferous vegetables cauliflower, cabbage, cress, bok choy, broccoli, and the spice mustard. The pollen was ingested with one of these plants, all of which have identical pollen. The small amounts of pollen could have been ingested with a spice made from mustard seeds. Generally, many more pollen grains are present in people who have eaten broccoli.

Fig. 4. Chichorieae pollen has an elaborate morphology. The pollen grain is tricolporate. There is a system of ridges with spines that radiate across the grain and around the pores and groove. This morphology is called fenestrate.

Small amounts of pollen grains from *Fagopyrum esculentum*, buckwheat, were found (Fig. 7). Buckwheat is a dietary type. Cultivation of buckwheat in Korea can be dated back more than 1000 years (Huh and Huh, 2000). Flour was used to make noodles. The relatively large and very distinctive pollen of buckwheat shows that this individual ate buckwheat in some form in the days before death.

Traces of pollen were recovered from several taxa. These probable dietary pollen types include the Apiaceae (carrot family), Caryophyllaceae (pink or carnation family), Solanaceae (tomato-chile pepper family). The Solanaceae family was restricted to Americas in prehistoric times and represents the introduction of economic plants, possibly chiles, to Korea from the Americas. Too few grains

of these types were found to identify the genera of the plants represented.

Discussion

Reinhard et al. (2007) argue that palynologists must be skeptical of their own identifications when interpreting pollen data, especially if they are working in a geographical area in which they have no experience. The most secure identifications in this analysis are rice, buckwheat, dandelion, Korean sage and mustard types. The yarrow-chrysanthemum identification is moderately secure to one of these two genera. Ethnobotanically, chrysanthemum has a greater history and diversity of use on the Korean peninsula. It may be necessary to use scanning electron

Fig. 5. This mint family pollen is distinct. One grain is imaged in polar view (left) and equatorial view (right). The pollen grain had six grooves that run between the polar areas. The surface is covered with a reticulum that is finer at the poles and between the grooves.

Fig. 6. Pollen aggregates are a strong indicator of intentional use of a plant. Here is a clump of three rice pollen grains. Like other pollen in the grass family, rice is spherical with a single pore (monoporate) and the pore has an annulus. The dimensions of cultivated grains, including the grain diameter and the annulus diameter, can be used to separate wild grasses from cultivated grasses.

microscopy to determine which genus is represented. The trace types are securely identified to family. Finding enough pollen grains of these traces to arrive at genus identification may be impossible, given the small sample that was submitted for analysis. The fact that chrysanthemum and dandelion are used in making types of wine suggested that these pollen types are evidence of wine consumption by the General.

The most challenging identification is that of the abundant bean family pollen. Identification of this pollen will be dependent on making a comparative reference collection of edible flowers in Korea. Previous experiments show that all pollen consumed by humans passes through the intestinal tract morphologically unaltered (Dean, 2006; Kelso and Solomon, 2006). The rate at which the pollen grains pass through the intestinal tract depends on the pollen type morphology. Transit time through the digestive system can vary from a few days to even weeks. Applying these observations to mummies, the pollen found in the intestinal tract could be consumed days or weeks before death. This is especially true of smaller pollen grains such as Brassicaceae, which can be passed as long as three weeks after the pollen is ingested (Dean, 2006). Large grains of cultivated grasses will pass for about a week (Dean, 2006). It is very likely that the buckwheat and rice pollen were ingested within six days before death. The other dietary types could have been consumed within two-three weeks of death.

Tens of thousands of grains from water plants, burweed or cattail, dominate the pollen spectrum. We believe that this was introduced with water. The large numbers of water-related pollen suggest that the General consumed broth, tea, or soup for a considerable time before death. Honey could also be a source for the insect pollinated types.

Determining the season of death in forensic palynology is based on comparing the pollen spectrum from the decedent with known seasonal pollen variation in the region where the decedent last lived. The seasonal variation of pollen in Korea has been recorded over several decades (Oh, 2009; Oh et al., 1998, 2012). The environmental

Fig. 7. *Fagopyrum* is a large and distinct pollen grain, tricolporate with pronounced reticulate surface.

pollen spectrum is composed of wind-pollinated plants in three categories: trees, weeds, and grass. The pollination for Seoul has been extensively studied (Oh et al., 1998, 2012). The main trees species that contribute to the pollen rain by month of pollination are alder (February-June), birch (February-July), poplar (March-July), hazelnut (March-July), maple (April-July), oak (March-July), and pine (February-August). Oh (2009; Oh et al., 2012), uses pollen concentration methods to estimate monthly pollen concentrations of wind pollinated types for several cities in Korea. They show that the period of pollination is different in Gangneung relative to Seoul. For Gangneung, only traces of pollen are released in February and March. Then, in April and May, tens of thousands of tree pollen grains are released per cubic meter of air. In June and July, only traces of tree pollen are released. The main trees species that contribute to the pollen rain by month of pollination are alder (March-May), birch (February-June), poplar (March-June), hazelnut (March-June), maple (April-June), oak (April-June), and pine (March-July). In Gangneung, grasses and weeds pollinate from April to the first part of November. The most abundant weed pollinators, sagebrush, and ragweed, are types that are easily

recognizable and preserve well archaeologically. The environment for Gangneung has been reconstructed by Park (2005) through palynological analysis of sediments for the past 740 years. Park reveals that around 260 years ago, the environment was changing from a pine forest to a pine oak forest that had a variety of trees. Therefore, we might expect even more arboreal pollen in the environment in which the general lived. The near absence of environmental pollen in the coprolite shows that death occurred after late November and before early February.

Conclusion

This Korean mummy provided very interesting intestinal food results in context of the paleopathological investigation. Lee and colleagues show that the General suffered a severe jaw injury some days before he died. The pollen indicated that he had eaten a diversity of foods in the weeks before death. These included rice, buckwheat, mustards, dandelion or chicory, mint relatives, bean relatives and others. The high quantity of wetland pollen from standing water plants suggests that in the days before death he ate broths or teas.

Comparisons were done to estimate the season of death. Using negative data in the form of absence of wild pollinated species, we conclude that the general died in winter between November and early February. There is more work to be done to identify the unknowns and refine identification of some of the more abundant types. There is a need to make comparative collections of edible bean flowers in Korea. Also, a reference sample of Korean Sage, *Agastache rugosa*, must be made to compare with the mummy's mint pollen. Scanning electron microscopy must be done to confirm the chrysanthemum identification. As research continues, even more information may be gleaned from the pollen of this fascinating individual.

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