Journal of Archaeological Science: Reports 21 (2018) 238–242

Contents lists available at ScienceDirect

Journal of Archaeological Science: Reports

journal homepage: www.elsevier.com/locate/jasrep

Pollen evidence of medicine from an embalming jar associated with Vittoria della Rovere, Florence, Italy

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ARTICLE INFO

Keywords:
Archaeopalynology
Embalming jars
Medicinal plants
Scanning electron microscopy
Myrtaceae
Italy
Medici

ABSTRACT

Various samples of human viscera fragments, sponges, and cloth were collected from embalming jars belonging to members of the Medici family of Florence. One jar was labeled with the name Vittoria della Rovere, who died in March of 1694. This jar contained viscera fragments that were identified as a section of collapsed intestine. The intestine of the Vittoria della Rovere sample contained a large concentration of pollen belonging to the Myrtaceae family. The Myrtaceae pollen was sometimes observed in clusters during analysis, which is indicative of purposeful ingestion of flowers, buds, or a substance derived from floral structures. Thus, the high concentrations and clustering of Myrtaceae pollen grains recovered from this sample are reflective of dietary or medicinal practices. Scanning electron microscopy indicated that the pollen was from cloves, *Syzygium aromaticum*. It is most likely that Vittoria della Rovere consumed cloves for medicinal or culinary reasons shortly before death.

1. Introduction

The Medici family of Florence, Italy, first came to power in the fourteenth century. Commerce and banking led to their rise of power and kept them in power for nearly three centuries. The family backed the ascension of four popes, giving them influence in all of Christendom and securing their sway beyond Florence. The remains of family members buried within the San Lorenzo Basilica, the church where the Medici Chapels are located, have been studied as part of a research project (Fornaciari et al., 2007; Giuffra et al., 2009). Several studies involving the health of the family through time have been completed since the project's beginning (Fornaciari et al., 2009; Giuffra et al., 2010). Further studies examined the embalming and autopsy techniques used in Italy during this time period (Giuffra et al., 2016). The entomological and arachnological examinations of the jar contents have been published previously (Morrow et al., 2016). The present study focuses on the recovery of pollen from an embalming jar that was also entombed within the San Lorenzo architectural complex. Pollen grains, presented here, are the most intriguing discovery from the jar.

The sample was one of 10 collected from embalming jars exhumed in 2010 from the Old Sacristy of the church, built in the fifteenth century. Analysis was done at the University of Nebraska-Lincoln in the Palynology Laboratory, School of Natural Resources and the Microscopy Core Research Facility, Center for Biotechnology. The jars had been used to collect materials used during the embalming process of members of the Medici family (Giuffra et al., 2016; Marinozzi and Fornaciari, 2005; Morrow et al., 2016). In 2011, samples from the jars were taken at the Department of Anatomy, Histology, and Forensic Medicine of the University of Florence, where the jars were temporarily stored prior to reinterment. During the 2011 sampling, labels were found on two jars, indicating specific family members associated with the jars' contents (Lippi, 2006). One contained the viscera of Anna Maria Luisa de' Medici, the last descendant of the Medici family, who died in February of 1743. The other contained the viscera of Vittoria

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https://doi.org/10.1016/j.jasrep.2018.06.039
Received 5 March 2018; Received in revised form 30 June 2018; Accepted 30 June 2018

della Rovere, the grandmother of Anna Maria Luisa de' Medici, who died in March of 1694. It is probable that some of the other jars also contained material associated with these two individuals. However, there were no labels on other jars to confirm their identities. This study focuses only on the analysis of the Vittoria della Rovere (VdR) jar.

2. Material and methods

The materials in each of the ten embalming jars were examined to determine the composition of the sample (Morrow et al., 2016). Some jars contained cloth and sponge remains used in the mummy preparation process, while others contained sections of intestine removed from corpses. The VdR sample consisted of intestinal tissue fragments. The analysis of this material is the focus of the present study.

The VdR sample was weighed and observations prior to rehydration were recorded. It weighed 2.91 g. It was then rehydrated using a 0.5% trisodium phosphate for approximately 48 h. Following rehydration, three *Lycopodium* tablets (Batch #124961; containing approximately 12,500 spores/tablet) were dissolved in HCl and then added to the rehydrated samples. The use of exotic spores to quantify pollen in ancient samples was developed by Stockmarr and is now standard with mummy studies (Piombino-Mascali et al., 2013; Reinhard et al., 2006, 2017; Stockmarr, 1971).

The 37,500 *Lycopodium* spores added to the 2.91 g sample from VdR equate to about 12,887 *Lycopodium* spores per gram of the sample. The sample was then disaggregated in a 600-ml beaker using a magnetic stirrer before being screened through a 250 μm mesh and rinsed with distilled water. The selection of a 250 μm mesh optimizes the separation of pollen and parasite remains. Macroscopic remains, primarily insects, were collected from the superior surface of the mesh while microscopic remains were screened through the mesh and into a beaker. Macroscopic remains were placed onto filter paper and allowed to dry before further examination. Microscopic remains were concentrated via repeated centrifugation and analyzed for the presence of mites, mite eggs, parasite eggs, starch granules, and other microfossils (Morrow et al., 2016). Following these analyses, acetolysis was employed for palynological investigations.

Processing samples using acetolysis is a common practice for pollen analysis (Piombino-Mascali et al., 2013; Reinhard et al., 2006, 2017). The process dissolves cellulose, chitin, and other materials, leaving primarily resilient microfossils, such as the sporopollenin of pollen grains. Acetolysis also darkens the pollen grains, which makes the pollen morphologically apparent. For this process, microscopic remains were transferred to a 50 mL centrifuge tube. They were washed with distilled water, and then centrifuged prior to decanting. This process was repeated with glacial acetic acid to prevent a reaction between the acetolysis solution and any residual water. The acetolysis solution was prepared as a 9:1 ratio of acetic anhydride to sulfuric acid. The solution was then added to the sample and once again vortexed to ensure that the residues were thoroughly mixed with the solution. Samples were then placed in a hot water bath of approximately 99 °C for ten minutes. After 10 min, the sample was centrifuged and the acetolysis solution was decanted into a hazardous waste container. The sample was then washed once with glacial acetic acid and subsequently washed multiple times with distilled water.

Following acetolysis, the material was transferred to a 2 dram archive vial using 95% ethanol and then glycerin was added for archival purposes. For analysis, drops of sediment from the sample were removed from the vial using an applicator stick. Drops were placed on a microscope slide, mixed with glycerin and then secured with cover slips. A compound microscope was used at 400× and 600× to perform a two hundred grain count over the span of three slides. *Lycopodium* spores were counted during this process so that an approximate count of pollen grains per gram of material could be determined. This was achieved using the following formula: pollen concentration = [(p / m) × a] / w, where *p* was the number of pollen grains counted, *m* was the number of marker grains (*Lycopodium* spores) counted, *a* was the number of *Lycopodium* spores added to the sample, and *w* was the total weight of the sample prior to rehydration (Piombino-Mascali et al., 2013).

Steps were taken to avoid contamination and maintain lab safety. Importantly, Myrtaceae flowers had never been processed in the lab prior to this analysis. Therefore, there could be no contamination from floral sources. Only sterile centrifuge glassware was used and all other equipment was cleaned thoroughly to ensure that no contaminants were introduced. The Palynology Laboratory is a filtered air, positive pressure, environmentally controlled facility that minimizes contamination. The lab's two research compound microscopes are Jenaval and Nikon instruments. The Jenaval compound microscope has differential interference contrast setting (DIC) and polarized light capability. It has 10×, 25×, 40×, and 100× objectives. The Nikon Eclipse compound microscope is designed for palynology, starch analysis, and parasitology. It has polarized light capability with 10×, 40×, 60×, and 100× objectives. It has image capture and analysis capabilities for bright-field and polarized settings.

For scanning electron microscopy (SEM), ~30 μl of the top layers of the prepared samples (in 100% ethanol) were pipetted and placed onto a 10 mm × 10 mm polycarbonate membrane (200 nm hole size) on a paper filter. After air-drying for 2–3 min, each of the membranes was placed onto a double-sided adhesive conductive tape on a SEM sample-mounting stub. The samples were further dried at 42 °C in a sample oven overnight before being sputter coated with a thin layer of chromium (~5 nm thick) using a Denton Vacuum Desk V sputter coater. Samples were examined on a Hitachi S-4700 Field Emission SEM and images were collected at different magnifications (1,000×–5,000×).

Morphometric analysis of pollen images from SEM was used to describe Myrtaceae species (Thornhill and Crisp, 2012; Thornhill et al., 2012a, 2012b). Specifically, one metric feature, the colpus length divided by overall length (C/L), was used for species determination. Images were analyzed by direct measurements on printed images and by digital measurement using Adobe Photoshop CC. Measurements were made only on images with pollen having an orthogonal orientation relative to the plane of focus, to avoid errors from perspective. Additionally, independent measurements of the same images were made by three individuals and averaged for analysis, to minimize any potential subjective measurement errors.

3. Results

3.1. Light microscopy

Most mummy intestinal and coprolite analysis features quantification through light microscopy. With LM, the pollen of Myrtaceae have long been recognized as easily recognizable at the family level (Erdtman, 1952). The Myrtaceae pollen concentration for the sample from this individual equates to 20,574 pollen grains per gram of intestinal remains. Other pollen types recovered in traces were *Pinus*, Poaceae, *Populus*, and *Castanea*.

3.2. SEM analysis microscopy

The genera and species within the Myrtaceae are difficult to identify without advanced microscopy. Using scanning electron microscopy, we were able to visualize the ultrastructure of the pollen. SEM analysis showed that the pollen grains featured scabrate sculpturing and were parasyncolpate, in other words, the colpi do not meet at the pole. Instead they form a triangular shape in the middle of the polar region known as the apocolpial field. Although it appears that apocolpial islands are present on some pollen grains, this may be an artifact of pollen grain distortion that led to elevated interior portions of the apocopial fields. It is worth noting here that both *Syzygium* and *Eucalyptus* are parasyncolpate. Of the three genera, *Myrtus* is distinct in