Mississippi Forensic News

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SPECIAL POINTS OF INTEREST:

- Welcome note from your new President
- 2022 Conference Info!
- Did someone say mummified chickens?!

INSIDE THIS ISSUE:

From the President

Greetings,

As a life member of the Mississippi Division of the IAI, I am excited to serve all current and future members this year as your newly elected president. The 2021 Conference, in Olive Branch, MS, was one of my all-time favorites. It was not because of the excellent crowd, the great venue, or even the awesome speakers; it was simply because I was delighted to see people and to fellowship with so many old and new friends.

Now that the world seems a little more normal, I am extremely excited about the upcoming spring conference this year in Hattiesburg, MS (date, time, and exact location to be determined). Hattiesburg is not only where I call home, but is home to a large number of our members as well. As the Hub City, Hattiesburg has long been recognized as a location which is central to many other sites in our region.

The past conferences in Hattiesburg have historically been some of the most well attended conferences, and we hope that does not change this year.

The mission of the MDIAI has always been to bring quality, professional, and educational material to our members as well as the law enforcement and forensic science communities. With that said, it is imperative to foster and mentor new students to the field. With Hattiesburg as the host site, it is home to William Carey University and The University of Southern Mississippi, two wonderful universities that both educate students in the field of Criminal Justice and Forensic Science. These universities will represent a large number of future practitioners that will one day be writing this same greeting letter.

The success of this organization has always been because of our great members. As we draw closer to the 2022 conference, please be thinking of how you can become involved. We are always looking for motivated individuals to help serve on committees and the board. If you are not a member, we encourage you to join our membership and to attend our conference.

In closing, I look forward to serving as president this upcoming year and welcome any questions, comments, or suggestions. The contact information for myself, the Officers, and the Board of Directors can be found on our website at www.mdiai.com.

See you in Hattiesburg!

Dr. Dean Bertram
MDIAI President 2021-2022

April, 2022

Check out Visit Hattiesburg for local attractions!

MDIAI invites you to Hattiesburg for our 2022 Annual Education Conference!

Stay Tuned!
More Details Coming Soon...

Important Information

Please visit our website to stay up to date on MDIAI information and our 2022 Conference!
https://www.mdiai.com/

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Need to renew your membership??
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We now take PayPal!

MDIAI was so happy to see everyone in Olive Branch! We missed you during Covid!

Conference Highlights

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Coming together is a beginning; keeping together is progress; working together is success

HENRY FORD

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Unraveling the Mummy: The Effects of Natural Mummification on the Recovery and Degradation of DNA

By Marissa Gandolfo-Muller, B.S.

ABSTRACT

This research focuses on observing and quantifying the differences in the recovery and degradation of postmortem DNA from specimens that have been naturally mummified. In this study, two control specimens and seven experimental specimens were placed in a variety of settings known to mummify tissue. After ten weeks, three of the specimens partially mummified, three specimens showed signs of superficial mummification and three specimens naturally decomposed. The specimens exposed to salt of neutral pH and cold temperatures, well known preservations of tissue and DNA, had greater DNA yield and lower rates of postmortem DNA degradation. The specimens exposed to UV radiation, alkaline pHs, and high temperatures showed lower DNA yield and higher levels of DNA degradation. The results of this research will make contributions to the fields of forensic identification and forensic anthropology, specifically, cold cases, victim identification in mass disasters and wars, and identification of genetic abnormalities within large gravesites through DNA analysis.

INTRODUCTION

Mummification has been known by cultures throughout the world for thousands of years. As defined by Piombino-Mascali et al., mummification is "the arrested decay by moisture loss and tissue desiccation" (2017, p. 101). The word "mummy" is derived from the Persian word *mumia*, meaning bitumen, which was used as a preservative in Egyptian mummies (Piombino-Mascali et al., 2017, p. 101). Mummification is a rare and varied biological process because it is a deviation from the body's natural decomposition cycle (Wieczorek & Rosendahl, 2010). The process of mummification can occur naturally or anthropogenically.

Although they have been found in cultures around the world, mummies are most popularly associated with Egypt. The ancient Egyptians are known throughout history for the elaborate tombs and the near-perfectly preserved mummies that inhabited them. While the ancient Egyptians are the most well-known practitioners of anthropogenic mummification, other ancient cultures also practiced the art of artificially preserving their dead. The Chinchorro mummies of northern Chile are the oldest known artificially mummified remains, predating Egyptian mummification by over 2,000 years (Wieczorek & Rosendahl, 2010). Other examples of ancient civilizations practicing anthropogenic mummification include Peruvian bundle mummies, Maori trophy heads, the living Buddhas of Japan, and Chinese wet mummies.

Much research has focused on anthropogenic mummification and inhibited decomposition cycles.

However, studies focusing on recreating types of natural mummification and analyzing the effects of natural mummification on DNA are limited to nonexistent. This study was undertaken to understand the natural mummification process and its effects on DNA recovery and degradation.

DNA begins to degrade soon after death as cells rupture, releasing nucleases that cause DNA to degrade into fragments over time (Rudin & Inman, 2002). The processes of autolysis and putrefaction, the two main components of an uninhibited decomposition cycle, can also accelerate DNA degradation (Pinheiro, 2010). In the decomposition cycle, autolysis is the destruction of cells, tissues, and organs by an aseptic chemical process and putrefaction is the process of decay caused by bacteria and fermentation (Pinheiro, 2010). In an uninhibited decomposition cycle, DNA has a half-life of 521 years (Allentoft et al., 2012). However, environmental conditions, such as time, temperature, humidity, light, and chemicals, influence the rate and degree of DNA degradation (Rudin & Inman, 2002). Ancient anthropogenic mummification processes can sometimes accelerate DNA degradation (Hawass et al., 2010). The modern embalming process introduces chemicals such as formalin into the body's tissues, which increases crosslinking in the DNA (Gielda & Rigg, 2017).

The purpose of this research is two-fold; first, this study recreates, as accurately as possible, the environments that allow specimens to naturally mummify in these recreated environments, and second, it examines the quantity and quality of DNA extracted. As such, the current study focused on two research questions:

Does natural mummification have a greater effect on postmortem DNA recovery and degradation than an uninhibited decomposition cycle?

What types of natural mummification, if any, increase the rate of postmortem DNA degradation?

METHODS

All the specimens were whole, organic chickens that were never frozen. This experiment consisted of nine specimens- two control specimens that did not undergo any type of degradation or mummification process and seven experimental specimens that each underwent a different kind of natural mummification. The control specimens were to undergo decomposition buried in soil and left in the open air. The seven recreated natural mummification settings were desert, air-based, rock salt, saline lake, frozen, cave-based, and bogs.

To begin analysis, the specimens were extracted from their recreated environments. Tissue samples were taken from the breast of each specimen except for the bog body specimen and the cave-based specimen, due to their state at the time of extraction from their recreated environments. The sample taken from the cave-based specimen came from the specimen's back and the sample taken from the bog body specimen was taken from any available tissue. Genomic DNA from the samples were extracted the same day the tissue samples were collected from the mummified samples and controls.

The three reagents prepared to use for the organic DNA extraction were stain extraction buffer, Tris-EDTA (TE) buffer, and Proteinase K. Genomic DNA was extracted in duplicate for each sample analyzed. The

organic extraction process was done three separate times, dividing the control and experimental samples into batches due to equipment constraints. After the organic DNA extraction process was completed, the quality and the approximate quantity of the genomic DNA was determined by processing the samples in 1% agarose gels containing ethidium bromide. To quantify the amount of DNA in the samples, a Thermo Scientific Nanodrop OneC Microvolume UV-Vis Spectrophotometer was used and the dsDNA setting was chosen to read the samples.

The Nanodrop indicated several samples as having a phenol impurity, so a second round of TE washings was carried out to remove the phenol. Then, a portion of the DNA samples was treated with RNase to remove RNA from the samples. After the RNase treatment and to remove any remaining RNase from the samples, ethanol precipitation of the DNA samples was carried out. The RNase treated, ethanol precipitated DNA samples were analyzed via gel electrophoresis and quantitated using the Nanodrop OneC spectrophotometer.

RESULTS

Mummification Results

Following the end of the ten-week observation period, all specimens were removed from their recreated environments. The specimens were separated into three categories: partially mummified, superficially mummified, and decomposed. The specific requirements for each category were derived from Pinheiro's research on the decomposition process of cadavers (2010) and Leccia et al.'s study on forensic mummies (2018). Each category used to describe the specimens is detailed in Table 1.

Table 1: Degrees of mummification

Partial Mummification	Superficial Mummification	Decomposition
• Dry and brittle skin • Desiccation of muscle tissue • Stiffness of extremities • Difficult to be dissected • Little to no fat present	• Dry and brittle skin • Stiffness of extremities • Putrefaction of muscle and/or fat • Decomposition of internal organs	• Dissolution of tissues to gases, liquids, and salts • Expulsion of internal liquids • Presence of mold and/or adipocere • Skeletonization

The mummification results of the specimens can be found in Table 2. Of the two specimens that were supposed to have undergone an uninhibited decomposition cycle, the soil decomposition specimen decomposed while the air decomposition specimen superficially mummified. Of the seven specimens intended to

mummify, five did. The desert specimen, bog body specimen, and saline lake specimen all partially mummified. The rock salt specimen and frozen specimen superficially mummified. The limestone cave-based specimen and the air-dehydration specimen decomposed.

Table 2: Specimen mummification results

Intended Outcome	Partial Mummification	Superficial Mummification	Decomposition
Decomposition	0	1	1
Natural Mummification	3	2	2
Total (n=9)	3	3	3

Each of the specimens had samples taken from their breast tissue, or in the case of the bog body specimen and air-based dehydration specimen, any tissue available. Each specimen was given a unique identifier and used in duplicate for whole genomic DNA extraction. The sample names and abbreviations are given in Table 3.

Table 3: Sample key

Specimen	Sample Label
Desert	D1, D2
Limestone cave-based	L1, L2
Rock salt	S1, S2
Air decomposition	AD1, AD2
Soil Decomposition	DD1, DD2
Bog body	BB1, BB2
Saline lake	N1, N2
Permafrost	P1, P2
Air-based dehydration	AB1, AB2
Control 1 (wing tip)	C1A, C1B
Control 2 (breast tissue)	C2A, C2B

DNA Analysis Results

After the organic DNA extraction process of the genomic samples, an agarose gel electrophoresis was performed to determine the quality of the DNA samples. The first gel contained 17 samples, eight in the first row and nine in the second row. The second gel contained eight samples, all in the first row. Figures 1 and 2 show the results of the organic DNA extraction process.

Figure 1: Gel 1 showing the DNA quantity and quality. As evidenced from the gel, the rock salt and permafrost treated samples had the highest DNA yield while desert, air decomposition and the saline lake specimens showed low DNA yield.

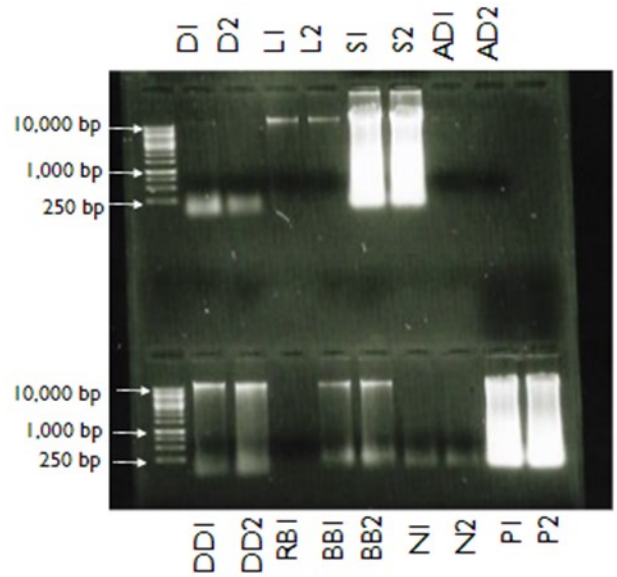
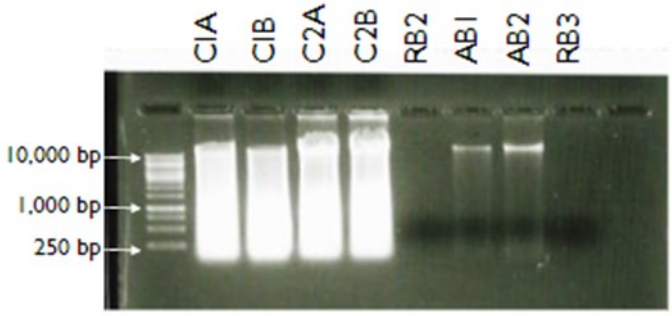


Figure : Gel 2 showing the DNA quantity and quality.



After the gel electrophoresis was performed, the samples were quantitated using the Nanodrop OneC spectrophotometer. The results of the gel electrophoresis and Nanodrop reading showed high amounts of DNA and RNA present in several samples. To remove the RNA from the samples, a portion of the DNA samples was treated with RNase.

The results of the samples after RNase treatment are shown in Figures 3 and 4. The samples after the

RNase treatment showed decreased levels of RNA, but the Nanodrop readings showed an increase, nearly double, in nucleic acid (ng/µL). The increase was most likely because of the increase in absorbance of DNA as well as the RNase enzyme (a protein).

Figure 3: Gel 1 DNA samples after RNase treatment. There is a considerable reduction in the RNA quantity (band in the 250 bp region). This data was supported by the reduction in DNA estimate through nanodrop.

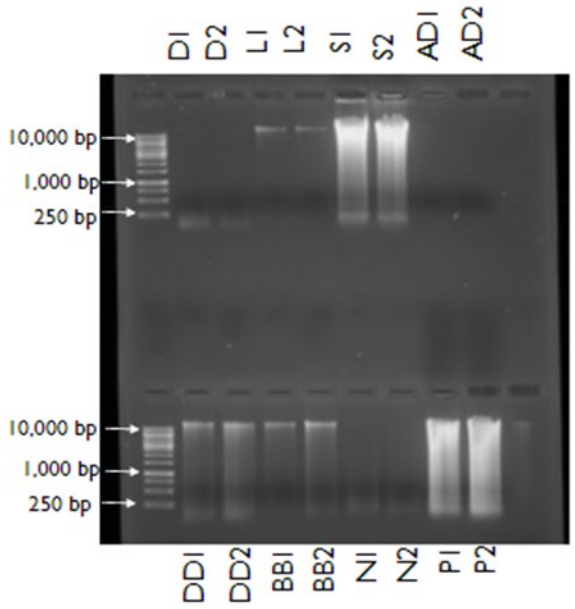
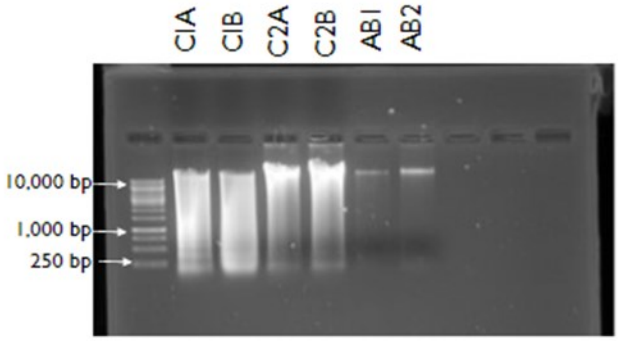


Figure 4: Gel 2 DNA samples after RNase treatment.



To remove the remaining RNase from the samples, ethanol precipitation of the DNA samples was carried out. After the ethanol precipitation, the samples were read with the nanodrop spectrophotometer for a final time. The results of the final nanodrop data supported the findings from the agarose gels, namely that there was a decrease in total DNA yield.

The highest yield of DNA was obtained from the rock salt and permafrost recreated conditions that other treated conditions. Despite partially mummifying, the specimen from the desert environment and the speci-

men from the saline lake environment showed the worst results of DNA preservation. The air decomposition specimen that superficially mummified also showed low levels of DNA quality and quantity. The quality of the DNA is measured by the degree to which a sample shows degradation and smearing in the agarose gel. A tight band near the gel wells shows good quality, while a smear shows degradation. The specimens that underwent the decomposition process, specifically the limestone cave-based specimen, the soil decomposition specimen, and the air-based dehydration specimen, showed a better DNA quality and quantity when compared to the partially mummified specimens. In conclusion, the results of the research show a significant relationship between certain types of natural mummification and their effects on the yield and degradation of postmortem DNA.

DISCUSSION

Previous research studies have shown that various forms of anthropogenic mummification, like those used for Egyptian and Chinchorro mummies, as well as modern embalming techniques can impede the recovery of DNA samples and accelerate the rate of degradation of postmortem DNA (Wieczorek & Rosendahl, 2010; Shved et al., 2014; Gielda & Rigg, 2017). The results of this research demonstrate that certain types of natural mummification also affect the recovery and degradation of postmortem DNA.

The DNA results from the rock salt specimen and permafrost specimen are supported by previous research that shows salt and freezing are two excellent preservatives for soft tissue (Piombino-Mascali et al., 2017; Shved et al., 2014). Wieczorek & Rosendahl cite freezing as the most efficient way to preserve the appearance of the body and its DNA (2010). The results of the DNA extracted from the desert specimen and saline lake specimen correlate with previous research and literature that states exposure to UV radiation, high temperatures, and highly alkaline pH are contributors to accelerated postmortem DNA degradation (Dean & Ballard, 2001). The air decomposition specimen most likely showed low levels of DNA due to the decomposition of its internal organs and growth of mold on its skin and interior chest cavity; microbes are known to accelerate the rate of decomposition and reduce the lifespan of DNA (Rudin & Inman, 2002).

In summary, the desiccation of tissue during mummification itself does not have an effect on the recovery and degradation of postmortem DNA, but the process through which the tissue desiccates has an effect on DNA recovery and degradation. The results of this research also demonstrate that for the best results of DNA recovery, quality, and quantity, retention of some liquid in the soft tissues is beneficial. The significant relationship found in this research was between environmental extremes and DNA degradation.

The results of this research will make contributions to several professional and academic fields. Within the field of forensic anthropology, results of this research could be applied to victim identification in mass dis-

asters and mass grave sites where bodies are found partially mummified, such as the mass grave sites from the Rwandan genocide. The analysis of the DNA extracted from ancient naturally mummified remains can make contributions to the field of paleopathology, the study of pathological conditions found in archaeological remains. DNA analysis of ancient remains could provide information on genetic malformations/abnormalities as well as the diseases our ancient ancestors suffered from.

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Special note- The above article represents an abbreviated version of the research as submitted to The University of Southern Mississippi by Marissa Gandolfo-Muller. If you are interested in more information, please email a request to **marissamuller2017@gmail.com** or message MDIAI at https://www.mdiai.com/contact

"The more that you read, the more things you will know. The more that you learn, the more places you'll go."— Dr. Seuss

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We ♥ our students!

The Mississippi Division of the IAI places a unique level of importance on the role of student members and the importance of student participation in our organization. Though the MDIAI places great importance on the professionals within our organization, the growth and development of young professionals and the provision of early opportunities for exposure to professional activities has been very important throughout the years. By providing positive growth opportunities in the formative years of young forensic professionals, MDIAI has been able to develop and maintain a core group of active, interested members who are sometimes more available to support and participate in activities than their senior counterparts who often have extensive obligations due to their age and stage, both personally and professionally.

Though occasionally some have raised issue with student access to sensitive information, this has rarely been an issue that impacts training or professional activities. The early and regular participation of young professionals has seemingly provided positive personal growth and similarly has positively impacted MDIAI over the years.

MDIAI would like to say a huge **THANK YOU** to all of the great professionals in Mississippi who selflessly serve our citizens! **BE SAFE!**