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

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by

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ABSTRACT

As demonstrated through casework and research studies (Hawass et al., 2010; Gielda & Rigg, 2017), anthropogenic mummification and modern-day embalming can expedite degradation of DNA. Current research in the field of forensic mummification is sparse and little research has been done on quantifying naturally mummified DNA (Leccia et al., 2018; Shved et al., 2014). This research focuses on observing and quantifying the differences in the recovery and degradation of DNA from specimens that have been naturally mummified. This research on natural, forensic mummies is a blend of experimental archeology and postmortem DNA analysis.

In this study, two control specimens and seven experimental specimens were used. Of the nine specimens, 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 could 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.

Keywords: natural mummification, experimental archeology, DNA analysis, DNA recovery, DNA degradation

DEDICATION

To my fellow scholars with unwavering passions for Egyptology, archaeology, and forensic science: death is only the beginning.

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LIST OF ABBREVIATIONS

B.C.E.	Before Common Era
BPB	Bromophenol Blue
DNA	Deoxyribonucleic Acid
dsDNA	Double Stranded DNA
EDTA	Ethylenediaminetetraacetic Acid
mDNA	Mitochondrial Deoxyribonucleic Acid
NamUS	National Missing and Unidentified Persons System
PCR	Polymerase Chain Reaction
PMI	Postmortem Interval
RFLP	Restriction Fragment Length Polymorphism
RNA	Ribonucleic Acid
SDS	Sodium Dodecyl Sulfate
TAE	Tris-acetic Acid EDTA
TE	Tris-EDTA
UV	Ultraviolet

CHAPTER I: INTRODUCTION

Since the early 1980s, DNA testing has become a staple in forensic analysis and the cornerstone of a solid conviction in a criminal investigation. This is because, with the exception of identical twins, every person has a unique genetic code, a unique DNA sequence (Rudin & Inman, 2002). However, 99.9% of human DNA is identical from person to person (Kobilinsky et al., 2005). The 0.1% that makes every individual unique does not sound substantial, but this variation of 1 base in every 1000 bases accounts for hair color, eye color, height, and ancestry characteristics (Kobilinsky et al., 2005; Rudin & Inman, 2002). In addition to physical characteristics, the unique 0.1% of DNA shows itself through blood type and genetic diseases (Rudin & Inman, 2002). This 0.1% difference between two human beings is the basis of forensic DNA testing.

DNA is fairly stable in a living organism, but as soon as a human being takes his or her last breath, their DNA starts to slowly degrade. An enzyme called nuclease breaks the phosphodiester bonds between the nucleotides, thus breaking the DNA strands into fragments (Butler, 2010). The decomposition cycle includes autolysis and putrefaction, two processes that can accelerate DNA degradation (Pinheiro, 2010). Natural factors like time, high temperatures, and chemicals with extreme pH can accelerate the degree and rate of DNA degradation (Rudin & Inman, 2002). Research and real-life cases have shown that anthropogenic mummification and modern embalming techniques while preserving the shape and aesthetics of the deceased's body, can chemically modify and degrade DNA (Hawass et al., 2010; Gielda & Rigg, 2017). Molecular biologists are increasingly developing new methods and technologies that improve the recovery of

damaged and degraded DNA, but there are still a number of settings in which it has not been fully investigated, one being naturally mummified organisms.

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:

1. Does natural mummification have a greater effect on postmortem DNA recovery and degradation than an uninhibited decomposition cycle?
2. What types of natural mummification, if any, increase the rate of postmortem DNA degradation?

The benefits of the study include adding to the lack of literature on the effects of natural mummification on postmortem DNA and making contributions to the fields of forensic identification and forensic anthropology.

CHAPTER II: LITERATURE REVIEW

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.

Mummification in Cultural Settings

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. The ancient Egyptians anthropogenically mummified their dead due to their religious beliefs about the afterlife. To the Egyptians, the human soul was split into three components: the *ba*, the *ka*, and the *akh* (Oakes & Gahlin, 2008). The *ba* was part of the spirit that was directly connected to the deceased's body but could leave the body and later return to it (Oakes & Gahlin, 2008). The *ka* was the deceased's double that embodied their moral character; the *akh* was equivalent to what Christians call the soul and entered the Duat, the Egyptian underworld, to receive judgment and possibly eternal life (Oakes & Gahlin, 2008). The Egyptians believed that, if the deceased's body were not properly embalmed and buried after the correct rituals or disfigured after death, then the deceased's spirit would not be recognized by the gods of the Duat and they would not have the opportunity

for eternal life (Oakes & Gahlin, 2008). By anthropogenically preserving the body, the Egyptians ensured eternal life for the deceased, both in the Duat and in history.

While the most famous Egyptian mummies belong to pharaohs and other members of the ruling class, animals were also mummified. The time-consuming, expensive embalming process was performed on cats, dogs, monkeys, bulls, crocodiles, and even snakes. Some Egyptologists postulate that deceased persons wanted to bring their pets with them into the afterlife and thus mummified them so they could spend eternal life together (Wieczorek & Rosendahl, 2010). Other Egyptologists posit that animals were mummified as part of religious practices. Many of the Egyptian deities had animal counterparts that were seen as physical manifestations of said deities by the Egyptian people (Oakes & Gahlin, 2008). Egyptologists believe that ancient Egyptians considered mummifying animals not only acted as an offering to their godly counterpart but as a way for the animals to reconnect with their deities in the afterlife (Wieczorek & Rosendahl, 2010).

From the beginning of the Third Dynasty in 2686 B.C.E. to the end of the Greco-Roman Era in the third century (Aufderheide, 2011), the ancient Egyptians practiced the art of mummification. The process began with extracting all the internal organs except the heart for preservation; the brain was removed through the nose and discarded (Aufderheide, 2011). According to the ancient Greek historian Herodotus, the body cavity was then washed out with wine and powdered spices before being stitched closed (Wieczorek & Rosendahl, 2010). The body of the decedent was then submerged in natron, a natural, salt-like mixture, to dry out the body's tissues (Aufderheide, 2011). After 70 days, the body was removed from the natron and any natron clinging to the skin

was lightly washed away (Oakes & Gahlin, 2008). The final step for embalmers was to wrap the body in strips of linen and place the body within its sarcophagus, or multiple sarcophagi set within each other if the decedent held a high status in society while alive.

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). In Chinchorro culture, embalmers would separate the head and extremities from the torso, remove the organs, sew the pieces back together with plant fibers, and then paint the mummy to resemble the deceased (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. Peruvian bundle mummies were made by removing the inner organs, heating the body over fire, and embalming it using organic resin (Wieczorek & Rosendahl, 2010). Maori trophy heads were severed from fallen warriors' bodies, stuffed with herbs that dried out the tissues, and then placed over a stove to dry (Aufderheide, 2011). These shrunken heads were a symbol of strength and courage in Maori culture but became popular curios with Europeans in the 19th century (Wieczorek & Rosendahl, 2010). The living Buddhas of Japan differ from other anthropogenic mummies because their mummification process began while they were still alive. The Buddhist monks would, over a period of three years, drastically reduce their caloric intake as well as ingest dehydrating substances; once the monks had died due to dehydration or starvation, their bodies would be dried out using heat or smoke

(Wieczorek & Rosendahl, 2010). Lastly, Chinese wet mummies underwent an in-depth chemical mummification process. The deceased would be bathed in alcoholic and astringent fluids, chilled over large bowls of ice, placed in a thick-walled wooden coffin lined with mercury, and buried in a mixture of kaolin clay and charcoal (Wieczorek & Rosendahl, 2010).

However, when analyzing anthropogenic mummies and their history, it is clear that embalmers, no matter the culture or civilization, learned from nature about how to preserve a body. Various environments can preserve bodies and create natural mummies as long as the conditions of the environment promote desiccation (Piombino-Mascali et al., 2017). Bogs, caves, deserts, lakes high in salt, and icy tundras can desiccate a body and lead to mummification (Wieczorek & Rosendahl, 2010). "Ginger", nicknamed for his red hair, is an Egyptian, predynastic mummy that was preserved by the hot desert, and dates back to 5,500 BCE (Rae, 1996). "Ginger" and the other Gebelein mummies, named for the location at which they were discovered, are considered precursors to the later established Egyptian process of mummification because those in charge of funerary rights observed how the desert sand desiccated tissues and maintained a recognizable form (Wieczorek & Rosendahl, 2010). Ötzi and the Children of Llullaillaco are examples of natural, frozen mummies, and the nearly impeccable preservation of their DNA and stomach contents serve as inspiration for modern cryonics (Piombino-Mascali et al., 2017; Wieczorek & Rosendahl, 2010). Ötzi was a Stone Age man who died a violent death and was preserved with his clothing and hunting equipment in the Alps for over 5,000 years (Piombino-Mascali et al., 2017). The Children of Llullaillaco were three Incan children sacrificed to their gods and entombed near the summit of Llullaillaco

volcano in Argentina in the 14th century (Wieczorek & Rosendahl, 2010). More examples of natural mummies include the Tollund Man, a bog body dating back to 280 BCE, and the Saltmen of Iran, miners trapped in a salt mine from a cave-in during the 4th century (Wieczorek & Rosendahl, 2010).

The Process of Mummification

In the field of forensics, present-day decedents' bodies can naturally mummify like the natural mummies of ancient history. The term "forensic mummies" has two meanings. According to Gitto et al., a forensic mummy is defined as a body that naturally mummifies in modern, man-made environments (2015, p. 53). Leccia et al. have a broader definition of a forensic mummy, describing it as mummified bodies found within the circumstances of a criminal or forensic investigation (2018, p. 1). Forensic mummies are most often discovered in enclosed areas with circumstances pointing toward signs of social isolation during the decedent's life (Gitto et al., 2015). Total indoor mummification is rare within the United States but is routinely encountered several times a year in Europe (Leccia et al., 2018). Occasionally, forensic mummies can be found outside, but total mummification is less likely to happen when compared to forensic mummies found indoors (Leccia et al., 2018). Mummification is more likely to occur indoors because of steady ventilation, little to no insect activity, and low moisture levels (Leccia et al., 2018; Pinheiro, 2010). Outdoor mummification commonly occurs in drier environments where the body's tissues can essentially dehydrate.

To fully understand the importance of forensic mummies, it is essential to understand how mummification occurs. Biologically, mummification begins when the processes of decay and putrefaction are impeded by the loss of moisture in the body's

soft tissue (Gitto et al., 2015). Soft tissues of the human body include fat, tendons, muscles, nerves, and blood vessels. Bacterial putrefaction is prevented when water is lost from soft tissues, because the various types of bacteria responsible for putrefaction favor hydrated tissue and humid air (Pinheiro, 2010). Extreme temperatures, osmosis, evaporation, and inhumation in soil high in salt content all promote water loss in soft tissue.

An individual's body factors also play a role in the mummification process. Low body weight, malnourishment, dehydration prior to death, and acute blood loss accelerate the process of mummification (Gitto et al., 2015). Skin lesions and burns can also accelerate the process (Pinheiro, 2010). Clothing and plastic on or wrapped around the decedent's body can have a moisture-wicking effect, pulling moisture out and away from the decedent's body (Leccia et al., 2018). The bodies of the elderly, children, and infants are more likely to mummify than those of adolescents and adults due to having thinner, less hydrated skin (Gitto et al., 2015; Pinheiro, 2010).

Despite the various conditions and causes of natural mummification, the appearance of mummified bodies is fairly uniform. Externally, the body's skin and soft tissues become dry and brittle, taking on a yellow-brown color and leathery texture (Gitto et al., 2015). Extremities and prominences of the body, like fingertips, toes, forehead, and cheekbones, are the first to desiccate (Pinheiro, 2010). Due to the dehydration and shrinkage of tissues and organs, the body undergoes significant weight loss (Wieczorek & Rosendahl, 2010). According to Pinheiro, it is common for minor adipocere to form during the mummification process because the water inside the body is used for the hydrolysis of fat to form adipocere, which accelerates desiccation of tissues (2010).

Adipocere, also called corpse wax or grave wax, is gray-white or yellow-brown and has a wax-like, crumbly consistency. Internally, organs shrink in size but maintain their shape and structure; preservation of internal organs allows for histological analysis (Gitto et al., 2015).

Because of the often long periods between time of death and when the decedent's mummified body is found, it is difficult to determine the time it takes for the mummification process to occur. In hot, dry environments, mummification can happen within two weeks outdoors or one to three months in an enclosed space (Pinheiro, 2010). The literature reports total mummification taking place in as little as two to three weeks but this rarely occurs within the forensic context due to the specific environmental conditions required for mummification (Gitto et al., 2015). Wet mummies, or bodies that mummify in moist environments, undergo a process some scientists call "corification". Corification describes the wet appearance of the desiccated tissue and the decomposition of the internal organs (Leccia et al., 2018). Wet mummies, which include bog bodies and ice mummies, can take a year or more to complete the mummification process (Lynnerup, 2015; Wieczorek & Rosendahl, 2010). Ice mummies fall into two categories: frozen and freeze-dried. Frozen mummies, considered "mummies" because of the preserved state of their tissues, maintain their water content and can begin or continue to decay if exposed to above-freezing temperatures (Pinheiro, 2010). Freeze-dried mummies are truly desiccated, with water frozen inside the body directly changing from a solid state to a gaseous one (Pinheiro, 2010). When it comes to most types of natural mummification, according to Gitto et al. (2010), adult decedents need 6 to 12 months to

complete the natural mummification process; children only need 3 or more months to fully mummify.

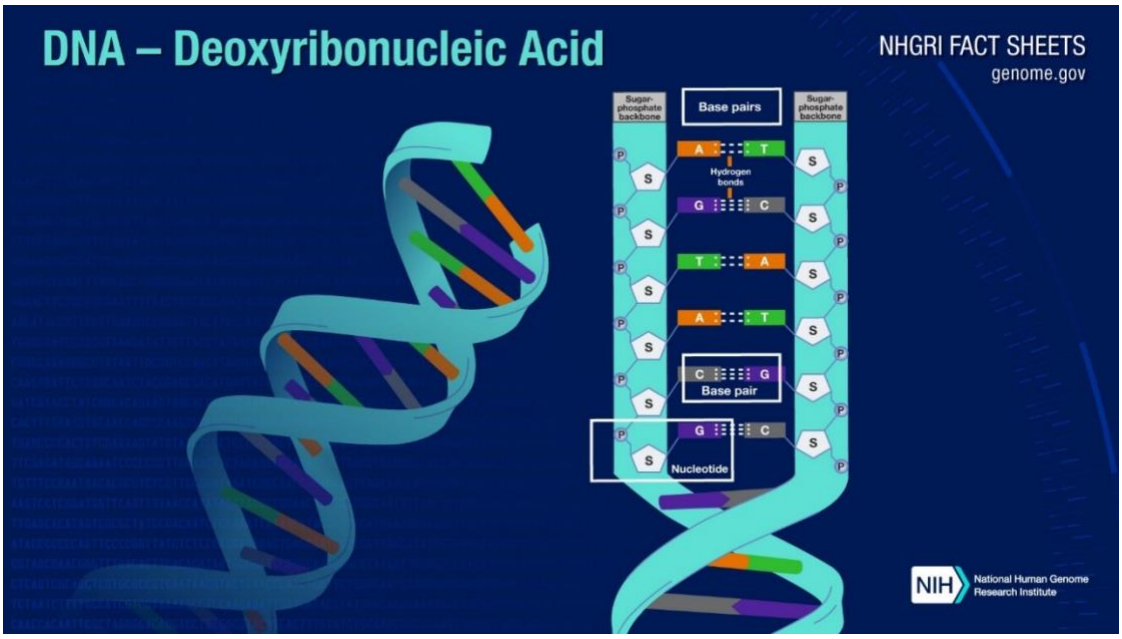
When first discovered, all mummies undergo an external examination as well as medical imaging, like x-rays and CT scan analysis. In the anthropological context, mummies are often subjected to carbon dating to determine the age of the mummy and isotope analysis via the mummy's hair to reveal diets, drug use, and environmental conditions during the decedent's lifetime (Wieczorek & Rosendahl, 2010). Mummies also have their DNA extracted to map or even sequence the human genome (Wieczorek & Rosendahl, 2010). Within the forensic context, mummified bodies may undergo autopsies, toxicology tests, histopathological analyses, and DNA tests to ascertain the cause of death, postmortem interval (PMI), and identity of the deceased (Leccia et al., 2018).

The Basics of DNA

Deoxyribonucleic acid, DNA for short, is the blueprint to all life. It contains the directions on how organisms develop, reproduce, and live. The building blocks of DNA are nucleotides, molecules made of a phosphate group, a 5-carbon sugar, and a nitrogenous base (Kobilinsky, Liotti, & Oeser-Sweat, 2005). The nitrogenous bases provide an organism with genetic variation while the phosphate group and 5-carbon sugar act as a structural backbone to DNA (Butler, 2010). The four nitrogenous bases are adenine (A), thymine (T), guanine (G), and cytosine (C) (Rudin & Inman, 2002). DNA has a double helix structure, commonly referred to as a ladder shape. The sides of the DNA ladder run antiparallel and are entwined around each other (Butler, 2010). The rungs of the ladder, called base pairs, maintain the DNA's structure. Base pairs are two

nitrogenous bases that are able form complementary base pairing such as A/T and G/C. Adenine (A) and Guanine (G) are classified as purines while Thymine (T) and Cytosine (C) are classified as pyrimidines (Butler, 2010). Purines can only bind to pyrimidines. This means that A binds to T via a double hydrogen bond and vice versa; G binds to C via a triple hydrogen bond and vice versa (Butler, 2010). Because of the structural differences between the nitrogenous bases, A can only bind to T and G only to C (Kobilinsky, Liotti, & Oeser-Sweat, 2005). The obligatory pairing between nitrogenous bases is referred to as complementary base pairing.

Figure 1: DNA Structure



Note. National Human Genome Research Institute, 2020

DNA is found in most cells within the human body, the exception being red blood cells. Within a cell, DNA can be found in two places: the nucleus and the mitochondria. The DNA found in the nucleus of a cell is referred to as nuclear DNA and is tightly packed into chromatin (National Human Genome Research Institute, 2020). The

chromosomes unwind during DNA replication and are transmitted from parent to child, creating the principle of hereditary (Rudin & Inman, 2002). The DNA found in a cell's mitochondria is called mitochondrial DNA, also known as mDNA. While humans inherit a half of their nuclear DNA from their fathers and the other half of nuclear DNA from their mothers, mDNA comes only from the mothers (National Human Genome Research Institute, 2020). mDNA only comes from the mother because during fertilization, only the woman's egg retains its mitochondria; the male's sperm does not (National Human Genome Research Institute, 2020).

Prior to assessing the quality and quantity of DNA, it must first be extracted from the cell. The most common types of DNA extraction are Chelex extraction, differential extraction, and organic extraction (Rudin & Inman, 2002). After the DNA is isolated, the DNA is examined for quality and quantity using a gel yield, slot blot, spectrophotometer, and/or species-specific quantitation methods. After examining the DNA sample's quality and quantity, the DNA is analyzed via either Restriction Fragment Length Polymorphism (RFLP) analysis or Polymerase Chain Reaction (PCR) amplification. RFLP analysis measures the sizes of DNA fragments located between designated restriction sites (Rudin & Inman, 2002). The advantages of RFLP analysis are its high reliability due to the precision of restriction enzymes and codominance, which allows analysts to differentiate homozygotes from heterozygotes (Kobilinsky, Liotti, & Oeser-Sweat, 2005). PCR amplification replicates a defined section of DNA millions of times, using the *Taq* polymerase enzyme to do so (Rudin & Inman, 2002). The advantages of PCR amplification include a faster turnaround time, the ability to use partially degraded DNA,

and requirement for only minute amounts of DNA, as low as one billionth of a gram (National Human Genome Research Institute, 2020).

Experimentation with Mummies and DNA

This research on natural, forensic mummies is a blend of experimental archeology and postmortem DNA analysis. Experimental archeology has been previously used to replicate artificial and natural mummification processes. Brier and Wade (1997) mummified a donated human specimen using a combination of salt and natron, recreating natural mummification caused by salt and the ancient Egyptian process of using natron to dehydrate and embalm bodies. Gill-Frerking and Healy used piglets from 2007 to 2009 in efforts to recreate bog bodies and examine the effects of highly acidic peat bogs on soft tissue versus bone (Gill-Frerking & Healey, 2011). There have also been numerous studies done on the natural decomposition of bodies (Wescott, 2018). However, there are no reports of experimental archeology being done to replicate natural mummification caused by soda/saline lakes, deserts, extreme cold, and dehydration, the most common type of mummification in a forensic context. There has also been no experiment-based research on how natural mummification affects postmortem DNA degradation, except for Shved et al.'s 2014 research on salt mummification using a human thigh. The present research intends to investigate DNA yield and degradation using the entirety of a chicken with specimens mummified in a variety of settings.

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, have an effect on the rate and degree of DNA degradation (Rudin & Inman, 2002). UV radiation and high salt concentrations are two of the greatest contributors to accelerated DNA degradation (Dean & Ballard, 2001). Some forms of preservation, like cryogenics, better preserve DNA and stall degradation, while other forms of preservation, like embalming, chemically modify or fragment DNA (Wieczorek & Rosendahl, 2010; Shved et al., 2014). 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).

In summary, much research has focused on anthropogenic mummification and inhibited decomposition cycles. Research focusing on recreating types of natural mummification and analyzing the effects of natural mummification on DNA is limited to nonexistent. This study was undertaken to understand the natural mummification process and its effects on DNA recovery and degradation. This research will provide supplementary data to the existing limited literature on DNA degradation caused by natural mummification.

CHAPTER III: METHODS AND MATERIALS

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. All the specimens were whole, organic chickens that were never frozen. Photographs of each specimen as well as weight, length, and width measurements, were taken prior to the decomposition or mummification process. All specimens, with the exception of the frozen natural mummification specimen and the bog body natural mummification specimen, were kept under the laboratory's fume hood. The fume hood was kept on 24 hours a day, for the entirety of the observation period.

The observation period was a ten-week period, in which the specimens were examined and the conditions were adjusted, if needed, at least once a week. Each week, photographs of each specimen and its environment were taken along with notes detailing changes to the specimen. Any adjustments in pH, sediment amount, or moisture levels were made during the weekly check-ins and recorded. The purpose of these adjustments was to most accurately replicate the natural environments in which the various types of natural mummification occur.

Recreated Natural Mummification Settings

Open Air Natural Decomposition Specimen

The specimen was placed in a 14 in x 12 in x 5 in container without a lid. Nothing was placed in the container except the specimen.

Soil Natural Decomposition Specimen

Two inches of a 14 in x 12 in x 5 in container was filled with topsoil. Making a small depression in the soil, the specimen was set on top of the soil. Another two inches of soil were added to fully cover the specimen. Every other week during the observation period, the soil was sprayed with tap water to emulate rain.

Desert Natural Mummification Specimen

Based on Seep's (2019) research on the composition of desert sand, natural calcium sand, rock salt, and silicon dioxide (diatomaceous earth) were mixed in a 4:1:1 ratio to fill three-quarters of a 10-gallon terrarium. Half of the sand mixture was used to line the bottom of the terrarium and to provide the specimen a "cushion" between it and terrarium bottom. The specimen was then placed into the sand mixture in the middle of the terrarium. The rest of the sand mixture was poured over the specimen to cover it. The final sand level was 5.2 in deep and weighed 9.6 kg. The terrarium's mesh lid was placed on top, and 13W UV dome lights were placed atop the terrarium lid. The dome lights were on 12 hours a day. Two terrarium heating pads were adhered to the terrarium; one was placed on the bottom side of the terrarium and the other on one side of the terrarium. The terrarium heating pads were used to keep the sand warm and dry. Any time the specimen was visible during the observation period, 280 g of the sand mixture, enough to fill a 500 ml glass beaker, was added to cover the specimen.

Air-based Natural Mummification Specimen

The specimen was wrapped in an organic cotton fabric, with as much skin covered as possible. The wrapped specimen was then placed in an airtight container

measuring 13.8 in x 10 in x 7.3 in. Nothing except the wrapped specimen was placed in the container.

Rock Salt Natural Mummification Specimen

Rock salt was used to line the bottom of a 13.8 in x 10 in x 7.3 in airtight container to provide the specimen a “cushion” between it and the bin's bottom. The specimen was then placed into the rock salt in the middle of the container. The rest of the rock salt was used to fully cover the specimen. The rock salt and specimen measured 7.75 in in height in the container. Next, 1.8 kg of rock salt was used to surround and cover the specimen. The lid was put on the airtight container, sealing it. Throughout the observation period, if the specimen was ever visible through the rock salt, 450 g of rock salt was added to cover the specimen.

Saline Lake Natural Mummification Specimen

Using 4 gallons of distilled water, a 10-gallon terrarium was filled three-quarters full. A 2:3 ratio of pure ocean salt and soda ash was added to the distilled water until the pH was within the 10 to 12 range. The total amount of pure ocean salt and soda ash mixture added to the water was 5.9 g. The initial pH was measured using a digital pH meter and was 11.06. The specimen was then submerged into the water-filled terrarium. If the pH of the water was not between 10 and 12 during the observation period, 1.2 g of the salt and soda ash mixture was added to increase the pH.

Frozen Natural Mummification Specimen

A 13.8 in x 10 in x 7.3 in airtight container was filled halfway with 3 in of topsoil. Making a shallow depression in the soil, the specimen was put into the soil. The specimen was then fully covered with more soil, for a total soil depth of 6.5 in. Then, 500

mL of tap water was poured over the soil. The container was then placed into a sub-zero freezer (-23°C). During weekly check-ins, the container was taken out of the freezer and allowed to defrost while checking on the other specimens in the laboratory. On alternate weeks, 500 mL of tap water was poured over the soil to emulate rain. Once photos and notes were taken on all the specimens, the container was put back into the sub-zero freezer.

Cave-based Natural Mummification Specimen

A pre-made mixture of dolomite/gypsum rock and pulverized limestone in a 2:1 ratio was used to line the bottom of a 13.8 in x 10 in x 7.3 in airtight container, providing the specimen a "cushion" between it and the bin's bottom, The total amount of dolomite and gypsum rock mixture and pulverized limestone used to line the bottom of the bin was 703 g. Making a shallow depression in the rock mixture, the specimen was placed onto the rocks in the middle of the bin. More of the dolomite/gypsum rock and pulverized limestone was used to cover the specimen. The total amount of dolomite/gypsum rock and pulverized limestone used to cover the specimen was 2.6 kg and the container was closed with a lid. The bin was wrapped in two lab coats to keep it in absolute darkness, or as much darkness as possible. If there was any point during the observation period that the specimen was visible through the rock, it was covered with the rock mixture.

Bog Body Natural Mummification Specimen

The bottom of a 24 in x 12 in x 12 in terrarium was lined with peat moss. The terrarium was filled three-fourths with 5 gallons of distilled water. Lactic acid powder and liquid humic acid were added in a 1:1 ratio until the pH of the water was within the 3 to 5 range. The total amount of powdered lactic acid added was 544 g. The total amount

of liquid humic acid added was 250 mL. Using a digital pH meter, the pH was found to be 3.84. The specimen was then submerged into the water-filled terrarium and more peat moss was laid atop the water. If the pH value of the water was not between 3 and 5 during the observation period, the previously stated amount of lactic acid and humic acid was added to lower the pH. Also, if the top layer of peat moss became submerged during the observation period, more peat moss was added.

Sample Collection

After the ten-week observation period was over, the specimens were extracted from their recreated environments. For each specimen, photographs were taken and extensive notes were taken to document the degree of mummification and any other visible changes to the specimen. With the exception of the bog body specimen, every specimen was weighed and the measurements of their length and width were taken. The weight and measurements of the bog body specimen were not taken due to the leatherization of skin and the dissolution of tissue and bone. Tissue samples were taken from the breast of each specimen with the exception of the bog body specimen and the cave-based specimen. 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.

DNA Analysis

DNA Reagents

The three reagents prepared to use for the organic DNA extraction were stain extraction buffer, Tris-EDTA (TE) buffer, and Proteinase K. The stain extraction buffer,

also called a lysis buffer, is a salt-based buffer solution that breaks open cells to allow for the analysis of their components (President's DNA Initiative, 2012). The stain extraction buffer used was prepared by adding 0.3 mg Tris base and 1.46 g of NaCl to a beaker. Using deionized water (diH_2O), the volume of the solution was brought to 150 mL. Then using hydrochloric acid (HCl), the pH of the solution was brought to 8.0. 25 mL of 20% Sodium Dodecyl Sulfate (SDS) and 5 mL of 0.5M Ethylenediaminetetraacetic Acid (EDTA) were added to the solution. The final volume was brought up to 250 mL with diH_2O.

The purpose of the TE buffer is to solubilize DNA, while also protecting it from further degradation while it awaits testing (President's DNA Initiative, 2012). To make the TE buffer, 1.21 g of Tris base and 0.037 g of EDTA were added to a beaker containing 800 mL of diH_2O. The pH of the solution was adjusted to 7.5 using hydrochloric acid (HCl). The final volume was brought to 1.0 L by adding diH_2O. The solution was then autoclaved and stored at 4°C.

Proteinase K inactivates the nucleases within a cell that would degrade DNA during a purification process (President's DNA Initiative, 2012). Proteinase K also digests and removes contaminating proteins from the sample (President's DNA Initiative, 2012). The Proteinase K used in these experiments was a 20 mg/mL stock solution made from 20 mg of Proteinase K powder and 1 mL of cold deionized water (diH_2O). The solution was then aliquoted (200 µL each) and stored frozen at -20°C until ready for use.

Organic DNA Extraction

Whole genomic DNA was extracted in duplicate for each sample analyzed. The extraction process was done three separate times, dividing the control and experimental samples into batches due to equipment constraints.

For each specimen, approximately 100 mg tissue were collected and weighed. In a 0.5 mL test tube, the tissue was ground with a disposable pestle with 100 µL of TE buffer and 100 µL stain extraction buffer. The ground tissue was then transferred to a 1.5 mL test tube and 300 µL of stain extraction buffer was added along with 10 µL of Proteinase K (20 mg/mL) for a total volume of 510 µL. The sample was incubated at 56°C overnight.

The next day, the sample was removed, given a quick spin with a vortex mixer to remove the condensate in the cap. Then, 500 µL of phenol-chloroform-isoamyl alcohol was added to each sample; the samples were vortexed gently to achieve a milky emulsion. The sample was centrifuged for 10 minutes at 12,000 rpm using an Eppendorf microcentrifuge. A MilliporeSigma™ Ultra Centrifugal Filter kit (Microcon, 100k) was assembled and labeled with the sample's number designation. After centrifugation, the aqueous phase was transferred to the Microcon kit. The Microcon filter kit was then centrifuged again at 5,000 rpm for 10 minutes. Once centrifugation was completed, the filter was removed from the kit, the flow through discarded, the filter placed back into the tube, and 500 µL of TE buffer added to the filter unit. The kit was centrifuged again at 5,000 rpm for 10 minutes. This process of washing was done for a total of five times.

After the TE washes were complete, the filter was removed, flipped into a new 2.0 mL test tube and centrifuged at 5,000 rpm for 5 minutes. Then, the filter was removed

and liquid at the bottom of the test tube was transferred to a new 1.5ml tubes. The DNA volume recovered from the Microcon was recorded and the sample stored frozen until analysis.

Gel Electrophoresis

The quality and the approximate quantity of the genomic DNA was determined by processing the samples in 1% agarose gels containing ethidium bromide. The 1% agarose gel was made by dissolving 1.0 g of agarose in 100 mL of 1xTAE buffer by boiling the agarose solution. The gel tray was placed in a gel casting tray, and the molten agarose was poured in the gel tray. One mm thick combs were used, and the gel was allowed to solidify for about 30 minutes.

Next, 2 µL of the genomic DNA samples were mixed with 2 µL of 5x Bromophenol blue (BPB) dye and loaded on to the gel. A Fermentas GeneRuler 1 kb DNA Ladder was used as a molecular size marker. The samples were electrophoresed at 100 volts for 20 minutes. Once electrophoresis was complete, the gel was transferred to a UV transilluminator and a picture taken for records.

Nanodrop Spectrophotometer

To quantify the amount of DNA in the samples, a Thermo Scientific Nanodrop OneC Microvolume UV-Vis Spectrophotometer was used. The dsDNA setting was chosen to read the samples; the samples were read at 260 nanometers (nm). The pedestals of the Nanodrop were wiped with a Kimwipe prior to loading the samples and in between each sample tested. Then, 1.5 µL of each sample was pipetted into the well. When the arm was closed, the Nanodrop OneC read the sample and the data was stored. Once all the samples had been tested, the data was extracted and saved to a USB drive.

Second TE Washing

For the samples that the Nanodrop indicated as having a phenol impurity, a second round of TE washings was carried out. The TE washing procedure outlined within the organic extraction procedure was used. Four additional TE washings were carried out to remove the phenol impurities.

RNase Treatment

In newly labeled tubes, 19 µL of genomic DNA was added. Next, 1 µL of the RNase enzyme (10mg/mL) was added to the tube for a final volume of 20 µL at a final concentration of 50 µg/mL. The tubes were given a quick spin in the centrifuge and gently vortexed. The samples were then incubated for an hour at 37°C. After an hour, the samples were removed and stored in the freezer until ready for use. The quality and quantity of the samples were analyzed by agarose gel electrophoresis and with the Nanodrop OneC spectrophotometer, respectively.

Ethanol Precipitation of DNA

To begin the ethanol precipitation, 84 µL of TE buffer was added to 16 µL of RNase-treated samples, bringing the volume to 100 µL. Then, 10 µL of 3.0 M sodium acetate and 275 µL of ethanol were added to the sample. The samples were vortexed gently and briefly spun in a microcentrifuge. The samples were incubated at -80°C for 30 minutes and centrifuged for 20 minutes at 4°C to pellet the DNA. The supernatant was poured off and the samples were washed with cold 70% ethanol. The samples were spun again at 4°C for 15 minutes. The supernatant was discarded and the samples were air dried at 37°C for 10 minutes. After air-drying, the pellet was then suspended in 16 µL TE buffer, to bring the volume of the sample to the starting volume. The samples were then

stored in the refrigerator until ready for use. The RNase treated, ethanol precipitated DNA samples were quantitated using the Nanodrop OneC spectrophotometer.

CHAPTER IV: 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 was also photographed, measured, and weighed. Table 3 shows the changes in weight the specimens underwent during the ten-week observation period. A majority of the specimens lost weight, with the air-based dehydration specimen losing the least amount of weight. The saline lake specimen was the only specimen that gained weight.

Table 3: Specimen weight changes

Specimen	Initial Weight (g)	Final Weight (g)	ΔWeight (g)
Desert	2622.0	1060.5	-1561.5
Limestone Cave-based	2291.8	1410.9	-880.9
Rock Salt	1773.1	1445.6	-327.5
Air Decomposition	2541.6	945.6	-1596.0
Soil Decomposition	1887.0	700.6	-1186.4
Bog Body	2505.0	Unable to be measured	Unable to be calculated
Saline Lake	2465.8	2870.8	+405.0
Frozen	2524.4	2258.7	-265.7
Air-based Dehydration	2067.3	2029.0	-68.3

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 4.

Table 4: 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 2 and 3 show the results of the organic DNA extraction process.

Figure 2: 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.

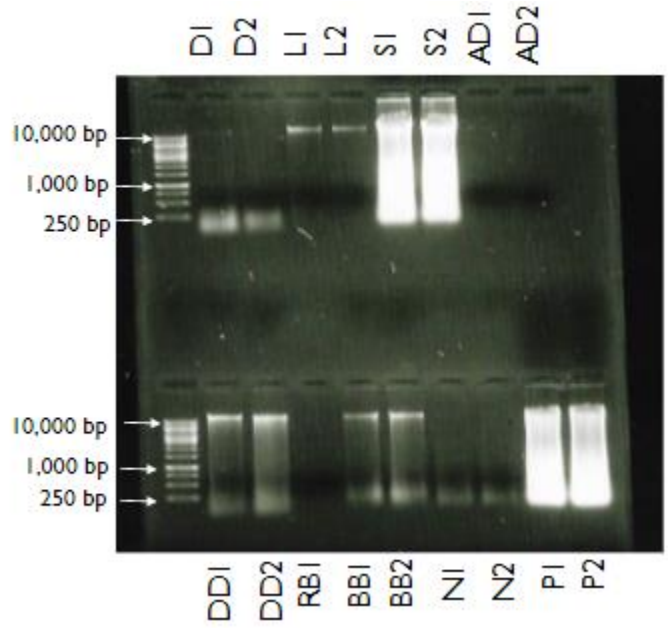
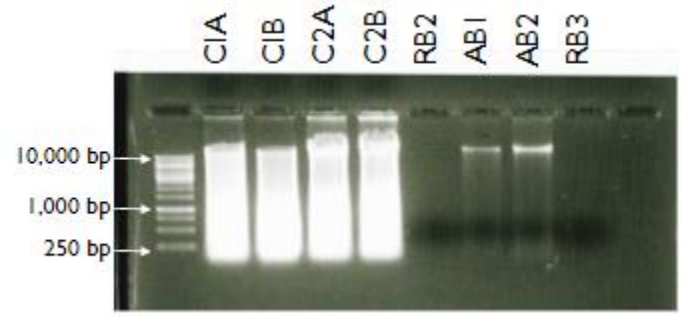


Figure 3: Gel 2 showing the DNA quantity and quality



After the gel electrophoresis was performed, the samples were quantitated using the Nanodrop OneC spectrophotometer. The complete results of the Nanodrop OneC reading are given in Appendix A. 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.

These samples were then analyzed through a 1% agarose gel and quantitated using a nanodrop spectrophotometer. The results of the samples after RNase treatment are shown in Figures 4 and 5. The complete results of the Nanodrop OneC reading are given in Appendix B. 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 in the nucleic acid portion of the Nanodrop reading was most likely because of the increase in absorbance of DNA as well as the RNase enzyme (a protein).

Figure 4: 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.

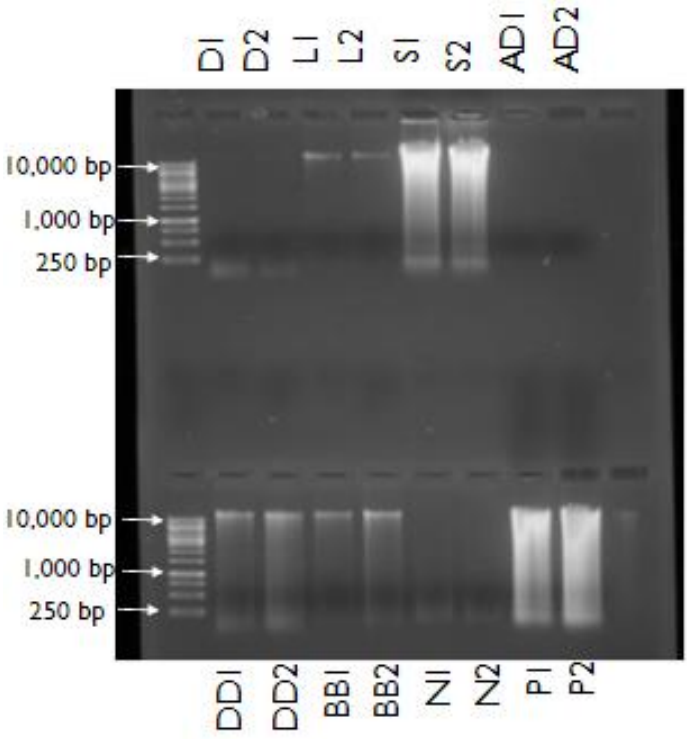
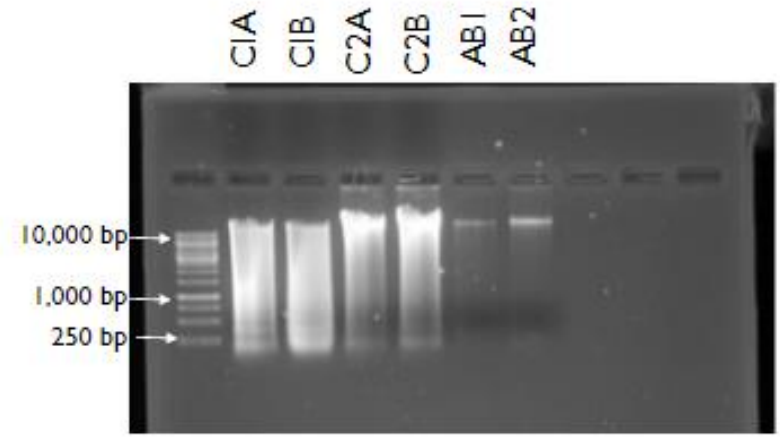


Figure 5: Gel 2 DNA samples after RNase treatment



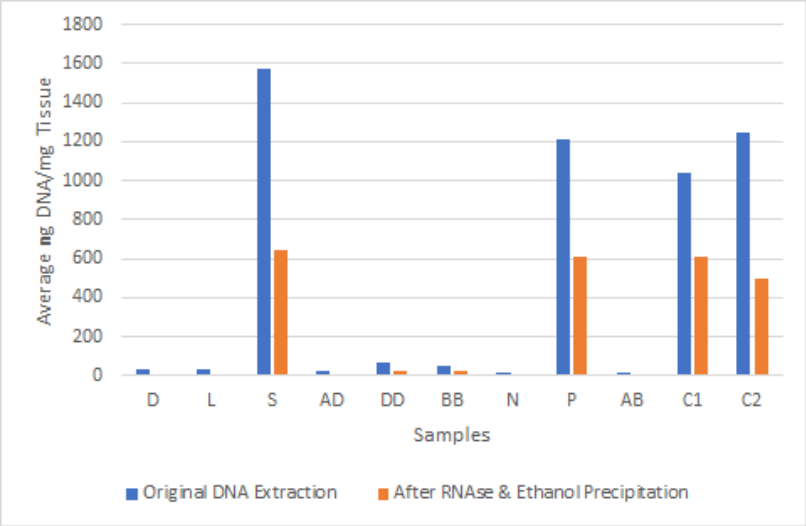
To remove the remaining RNase from the samples, ethanol precipitation of the DNA samples was carried out. After the ethanol precipitation, the samples were read with the nanodrop spectrophotometer for a final time. The results of the final nanodrop data supported the findings from the agarose gels, namely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

Calculations were performed to estimate the total DNA (ng) recovered from the tissues, total DNA (ng/mg tissue weight), and the average total DNA (ng/mg tissue weight) for each specimen. To calculate total DNA (ng), the Microcon DNA volume (µl) was multiplied by the nanodrop quantitation data (ng/µl). The total DNA (ng/mg tissue weight) was calculated by dividing the calculated total DNA (ng) by the mg tissue weight used for DNA extraction. The histograms in Figures 6 and 7 show the average total DNA (ng/mg tissue weight) for each specimen.

Table 5: Final DNA yield calculations

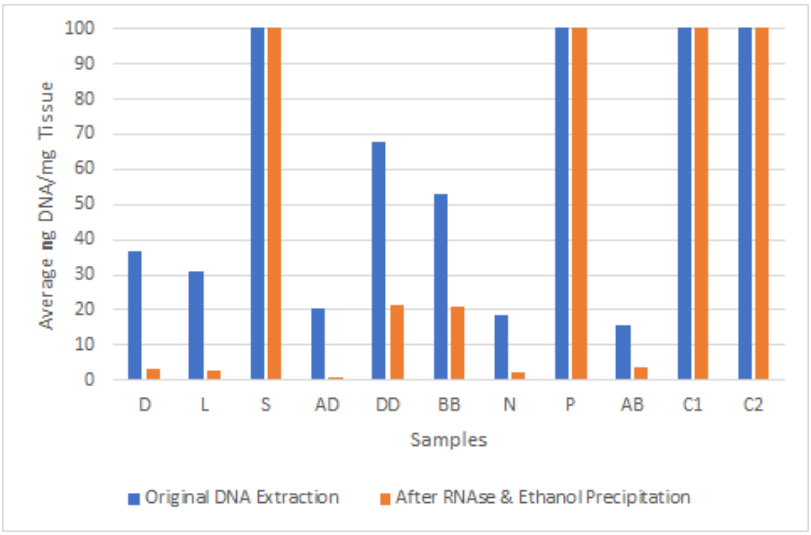
Sample	Tissue Weight (mg)	Microcon DNA Volume (µl)	Nanodrop Quantification (ng/µl)	Total DNA (ng)	Total DNA (ng)/mg Tissue Weight
D1	108.8	47.0	11.5	542.6	4.9
D2	86.4	44.0	3.9	171.2	1.9
L1	76.7	47.0	5.3	247.6	3.2
L2	78.4	48.0	3.7	176.7	2.3
S1	86.3	45.0	1206.3	54282.8	629.0
S2	89.2	46.0	1269.1	58376.7	654.4
AD1	84.4	44.0	1.9	85.8	1.0
AD2	74.6	49.0	0.6	31.6	0.4
DD1	76.3	43.0	27.9	1200.0	16.6
DD2	71.1	51.0	35.9	18.4	26.0
BB1	101.1	42.0	19.9	836.0	8.4
BB2	104.2	47.0	54.8	25.8	25.0
N1	96.7	48.0	5.9	282.0	2.9
N2	98.8	46.0	4.2	193.8	1.9
P1	97.3	49.0	1080.8	52961.1	544.3
P2	94.4	52.0	1220.1	63445.5	672.1
AB1	102.7	48.0	5.3	256.4	2.5
AB2	98.2	44.0	12.1	533.3	5.4
C1A	93.0	51	1076.3	54890.9	590.2
C1B	109.0	75	904.4	67830.7	622.2
C2A	108.0	55	799.5	43972.3	407.2
C2B	96.0	57	978.5	55774.2	580.98

Figure 6: Summary of DNA recovery results



Key:
D: Desert
L: Limestone cave-based
S: Rock salt
AD: Air decomposition
DD: Soil decomposition
BB: Bog body
N: Saline lake
P: Permafrost
AB: Air-based dehydration
C1: Control 1 (wing tip)
C2: Control 2 (breast tissue)

Figure 7: Same data given in Figure 6 but this graph has an expanded y axis to visualize the low-level DNA in some samples



Key:
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DD: Soil decomposition
BB: Bog body
N: Saline lake
P: Permafrost
AB: Air-based dehydration
C1: Control 1 (wing tip)
C2: Control 2 (breast tissue)

The highest amount 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 specimen 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 recovery and degradation of postmortem DNA.

CHAPTER V: DISCUSSION

The purpose of this research was to analyze the effects various forms of natural mummification have on the yield and degradation rates of postmortem DNA. 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 do 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).

Shved et al. (2014) and Lombardero et al. (2017) completed similar research to this study, but solely focused on the effects of salt on tissue preservation and DNA

degradation. The results of those research projects also demonstrate that salt is a preservative for both tissue and DNA. The results of Gill-Frerking & Healey's (2011) research with bog bodies shows that soft tissue is excellently preserved and Wieczorek and Rosendahl point out that several ancient bog bodies have had their DNA extracted and successfully amplified (Wieczorek & Rosendahl, 2010).

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.

CHAPTER VI: CONCLUSION

Based on the findings of this research, and in light of previous research done, it can be concluded that it is how the specimen's tissue desiccates, not overall natural mummification, that affects the recovery and degradation of postmortem DNA. The specimens exposed to salt of neutral pH and cold temperatures, both well-known preservatives of tissue and DNA, had greater yields of DNA and lower rates of postmortem DNA degradation. The specimens exposed to UV radiation, alkaline pHs, and high temperatures showed lower rates of DNA recovery and higher levels of 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 disasters and mass grave sites where bodies are found partially mummified, such as the mass grave sites from the Rwandan genocide (Longman, 2019). 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 (Anastasiou & Mitchell 2013).

However, the findings of this research would most benefit the field of forensic identification. Recently, the National Missing and Unidentified Persons System (NamUs) has begun working with state and federal crime labs to identify bodies found partially mummified and skeletonized in the deserts of the American Southwest. Knowing from which parts of the body to collect samples and how much DNA to expect within a sample

from a naturally mummified body will save agencies time and money when identifying unidentified and unclaimed bodies. While the mummified bodies in the American Southwest are a specific example, finding mummified bodies is common in Europe due to their climate. Furthermore, mummification is often a sign of social isolation or familial abandonment. Also, if a reference DNA profile is not available to compare with that of the unknown DNA profile obtained from the extracted samples, law enforcement agencies can use the extracted DNA to look at past medical history to narrow down a list of possible identities. Finally, on a broader spectrum, this research can fill the current gap in the literature on the relationship between DNA degradation and natural mummification.

Evaluating the research design, there are some improvements that could be made: Observations could have been made daily instead of weekly, the decomposition specimens could have been kept outside during the observation period, and a sample could have been taken from the soft tissue and bone of each specimen. If there had not been time and monetary limitations to this research, more types of natural mummification, such as tar and volcanic ash, and more than one specimen per type of natural mummification would have been observed. Steps that could have been taken within the DNA analysis process extend to amplifying, if possible, the DNA extracted from the specimen.

The most important suggestion for future research is to allow the specimens to mummify or decompose in their environments for as long as possible. This way the effects of the various environmental conditions are not only being tested but also the effect of time on DNA recovery and degradation. Other suggestions for future research

include preparing soft tissue for histological analysis and using human tissue specimens instead of animal specimens.

In conclusion, the results of the research show a significant relationship between certain types of natural mummification and their effects on the recovery and degradation of postmortem DNA.

APPENDIX A: NANODROP READING AFTER ORGANIC EXTRACTION

Sample Name	Nucleic Acid(ng/uL)	A260/A280	A260/A230	Corrected (ng/uL)	Impurity
D1	88.367	1.64	1.886	52.65	Phenol
D2	68.535	1.568	1.816	31.6	Phenol
L1	63.27	1.44	1.805	0	Phenol
L2	37.766	1.365	1.353	0	Phenol
S1	3041.172	2.007	2.156		
S2	3017.298	2.02	2.032		
AD1	35.763	1.306	1.586	0	Phenol
AD2	34.184	1.315	1.694	0	Phenol
DD1	87.017	1.58	0.902	55.7	Phenol
DD2	121.208	1.647	1.024	89.2	Phenol
RB1	30.007	1.246	1.746	0	Phenol
BB1	82.856	1.665	1.444		
BB2	157.659	1.599	1.244		
N1	37.117	1.36	1.506	0	Phenol
N2	41.092	1.342	1.445	0	Phenol
P1	2152.773	2.033	2.271		
P2	2439.369	2.039	2.28		
AB1	22.015	1.347	1.449	0	Phenol
AB2	47.399	1.525	1.544	23.6	Phenol
RB2	15.024	1.166	1.684	0	Phenol
C1A	1949.005	2.021	2.313		
C1B	1472.024	1.984	2.176		
C2A	2695.618	2.038	2.285		
C2B	1891.888	2.033	2.275		
RB3	-6.986	1.902	1.946		

APPENDIX B: NANODROP READING AFTER RNASE TREATMENT

Sample Name	Nucleic Acid(ng/uL)	A260/A280	A260/A230	Corrected (ng/uL)	Impurity
D1	48.923	1.502	0.864		
D2	36.354	1.494	0.827		
L1	37.095	1.577	0.655		
L2	21.32	1.599	0.589		
S1	4250.603	1.839	2.138		
S2	3228.137	1.805	2.007		
AD1	13.255	1.481	0.422		
AD2	9.317	1.315	0.406		
DD1	66.604	1.666	0.694		
DD2	152.17	1.657	0.806	137.95	Protein
BB1	142.37	1.649	1.178		
BB1	246.891	1.61	1.161		
N1	15.599	1.443	0.475		
N2	19.681	1.408	0.442		
P1	2659.936	1.851	2.183		
P2	2690.014	1.867	2.194		
C1A	3163.425	1.812	2.189		
C1B	2353.465	1.838	2.112		
C2A	2394.127	1.834	2.172		
C2B	2342.087	1.835	2.192		
AB1	28.647	1.588	0.581		
AB1	54.142	1.633	0.732		

APPENDIX C: NANODROP READING AFTER ETHANOL PRECIPITATION

Sample Name	Nucleic Acid(ng/uL)	A260/A280	A260/A230
D1	11.545	1.759	2.225
D2	3.891	2.108	2.085
L1	5.268	1.539	2.322
L2	3.681	1.517	1.361
S1	1206.285	1.87	2.553
S2	1269.058	1.873	2.532
AD1	1.95	1.732	1.433
AD2	0.646	1.524	4.315
BB1	19.912	1.575	1.05
BB2	54.832	1.533	0.943
DD1	27.915	1.696	0.799
DD2	35.99	1.747	0.937
N1	5.875	1.902	1.99
N2	4.213	1.681	2.491
P1	1080.84	1.87	2.513
P2	1220.106	1.873	2.534
C1A	1076.292	1.853	2.423
C1B	904.409	1.842	2.219
C2A	799.497	1.85	2.398
C2B	978.495	1.847	2.454
AB1	5.343	1.664	5.483
AB2	12.121	1.771	1.592

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