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Ritual and politics: exploring human sacrifice at Midnight Terror Cave, Belize

Author
Verdugo, Cristina

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RITUAL AND POLITICS: EXPLORING HUMAN SACRIFICE AT MIDNIGHT TERROR CAVE, BELIZE

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By

Cristina Verdugo

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The Dissertation of Cristina Verdugo is approved:

Professor Lars Fehren-Schmitz, Chair

Professor Alison Galloway

Professor James E. Brady

Chelsea Blackmore, Ph.D.

Quentin Williams
Acting Vice Provost and Dean of Graduate Studies

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ABSTRACT

Ritual and Politics: Exploring Human Sacrifice at Midnight Terror Cave, Belize

By

Cristina Verdugo

My dissertation research explores the relationship between human sacrifice and power by examining the recruitment of sacrificed individuals utilizing the Midnight Terror Cave (MTC) skeletal collection, the largest sacrificial assemblage in the southern Maya lowlands. Early anthropological studies of human sacrifice focus heavily on ritual and beliefs. In contrast, this dissertation is innovative by ignoring the emic rationalization of sacrifice to construct a model utilizing elements from ritual violence and performance theory. This approach explores the complex links between power, politics, and sacrifice. Sacrifice by its very nature is a political act as it asserts the state's right to take human life. However, it must be played out in relation to differentials of status due to gender, lineage, and age. Ritual is used to deflect possible resistance but with priests or the elite as central actors, it is clearly legitimizing power structures under the guise of reviving or sustaining the existing social order.

This research reconstructs who was sacrificed by combining paleogenomic, osteological, ethnohistoric, and archaeological approaches. The analysis of the MTC material produced demographic data for individuals interred in the cave. My results suggest that females may have been selected more frequently than males and another study of the MTC assemblage shows that nearly half of the individuals are subadults.

This clearly contradicts the idea that victims were recruited through warfare as it is usually portrayed. In addition, this dissertation outlined the evidence for human sacrifice as opposed to burial at MTC. In doing so it became evident that osteological remains in Maya caves are most likely associated with human sacrifice.

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CHAPTER 1

Introduction

Public interest in the ancient Maya was sparked by Stevens and Catherwood's drawings of Copan in the late 1880's. Since then, our understanding of ancient Maya life has deepened through archaeological investigation. The Maya area is defined as the territory where the 26 or so Maya languages are or were spoken at the time of the Spanish Conquest (Sharer and Traxler, 2006). Geographically, it includes the modern states of Guatemala and Belize, the Mexican states of Tabasco and Chiapas, all of the Yucatan Peninsula, as well as the western portions of Honduras and El Salvador (Figure 1). At its height in the Classic Period (250-900 CE), the Maya region was divided into more than 60 kingdoms (Martin and Grube, 2000) with large centers at sites such as Tikal, Chichen Itza, Naranjo, and Caracol. The ancient Maya are known for their unique writing system, numbering system, calendrical system, architecture, art, religion and ideology, as well as their complex economy, social, and political systems (Sharer and Traxler, 2006).

Figure 1. Map of the Maya area. Courtesy of www.latinamericanstudies.org

The ancient Maya practice of human sacrifice has also drawn attention since the dredging of the Cenote of Sacrifice at Chichen Itza by Thompson from 1904-1910. Until recently, human sacrifice was not considered as an explanation for the presence of skeletal remains in caves. Caves containing human remains were proposed as ossuaries, cremation, or ancestral worship locations (Blom, 1954; Thompson, 1975). These studies, primarily focused on Mexico, note post-mortem modification, limited number, and deliberate placement or organization of remains. More recently, some scholars discount the practice of human sacrifice by the ancient Maya and instead argue for use of caves as elite burial spaces (Wrobel et al., 2014). Clearly, the link between human remains found in cave space and the practice of human sacrifice is still debated.

Hypothesizes for the ancient Maya practice of human sacrifice vary by sex, age, socioeconomic status, and local vs non-local status (Ambrosino, 2003; Anda Alanís, 2007; Barret and Scherer, 2005; Beck and Sievert, 2005; Duncan, 2011; Geller, 2012; Houston and Scherer, 2010; Lucero and Gibb, 2007; Massey and Steele, 1997; Prout and Brady, 2018; Saul and Saul, 1989; Scherer, 2017; Tiesler 1998, 2007; Tiesler and Cucina, 2007; Tozzer, 1944). Early accounts outline the sacrifice men, women, and children in the Cenote of Sacrifice (Tozzer, 1944). Since then, discussions have ranged from the sacrifice of virgin women to the sacrifice of male war captives (Arnold and Frost, 1909; Demarest et al., 1997). Given the dearth of sacrificial assemblages, MTC provides an opportunity to re-examine these hypotheses using new methodologies which can provide demographic well as genetic data for sacrificed individuals.

This dissertation research assesses the Midnight Terror Cave (MTC) skeletal population and its role in ancient Maya human sacrifice. This collection, consisting of over 10,000 skeletal elements, constitutes the largest cave assemblage from the southern lowlands attributed to sacrifice. The discussion of human sacrifice has taken a strange trajectory in the Maya area and widely accepted popular views, while never endorsed in scientific circles, have nevertheless had an impact on archaeology. Varying sources coupled with a dearth of skeletal data has created conflicting theories regarding the sacrifice of men, women, and children. As a result, the question of who was sacrificed has continued to persist in archaeological discussions.

This research has resulted in a “hybrid” dissertation composed of 3 published articles, a discussion, and a concluding chapter (outlined below). In their own ways, these chapters tackle questions related to the demographic composition of the MTC remains as well as evidence for the practice of human sacrifice at MTC. This research combined paleogenomic and osteological analyses in order to extract sex, age, or ancestry data from the bone. The additional data provided by paleogenomic analysis often allows this work to transcend barriers encountered by traditional osteological analysis. Results of this analysis produced new insights into sacrificial practices, clarifying questions and materially advancing our scholarship.

1.1 Midnight Terror Cave

MTC located in the Cayo District of Belize, was discovered when a looter fell 30-40 feet off the cave ledge requiring rescue from the nearby community (see chapters 3 and 4 for map). The MTC project ran from 2008-2010 as part of the Western Belize Regional Cave Project directed by Dr. Jaime Awe. Survey of the cave was carried out by California State University, Los Angeles (CSULA), under the supervision of Dr. James Brady. The project was terminated in 2010 when CSULA ceased supporting a Summer Quarter which had permitted Brady to work during the dry season (Spring Quarter). It was felt that surveying the cave during the rainy season was too dangerous so many planned investigations were never carried out.

The cave is located within half a kilometer of the major site of Tipan Ch’en Uitz (Figure 2). According to Andres and Wrobel (2011), the presence of formal, large scale architectural modification around and in MTC suggests that the cave was controlled by Tipan Ch'en Uitz. Architecture in form of masonry platforms and mounds were found on top of the hill in which MTC is located, but the mapping and excavation of this area was beyond the scope of the project. A plazuela group at the base of the hill also suggests that this area may have been part of the larger Tipan Ch'en Uitz settlement.

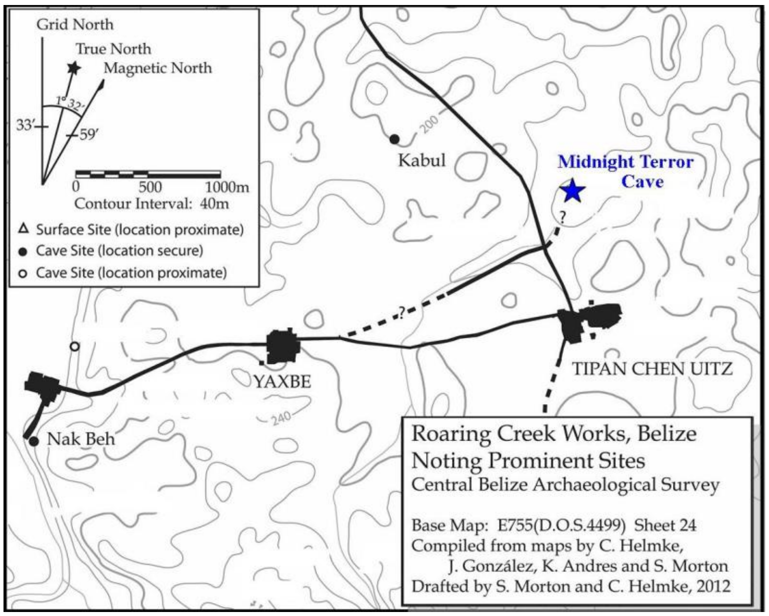

Figure 2. Map showing MTC and Tipan Ch'en Uitz (Map after Morton and Helmke 2012).

The cave entrance is situated near the top of the hill with a large earthen platform creating level space in front of the entrance. MTC is approximately 250 meters in length and consists of two chambers. During the investigation, the cave was broken down into eight 'Operations' based on natural boundaries in order to provide greater spatial control (see chapter 3 for map). From the entrance, the descent into the cave is negotiated along slippery ledges (Operations I-III) to the floor of the cave (Operation IV) some 20 m below. Along the pathway in Operation III, archaeologists discovered a series of altars had been erected, with the remains of offering scattered around them. Architectural modifications within MTC include two, large constructed plazas in Operations IV and VII, and two leveled areas surrounded by terraces in Operation V (Figure 3). Each of these constructions created large, level spaces for conducting public rituals. Additionally, a network of constructed pathways connects the three areas, creating a formalized circuit which directed visitors through the cave (Figure 4).

Figure 3. Terraces located in Operation V.

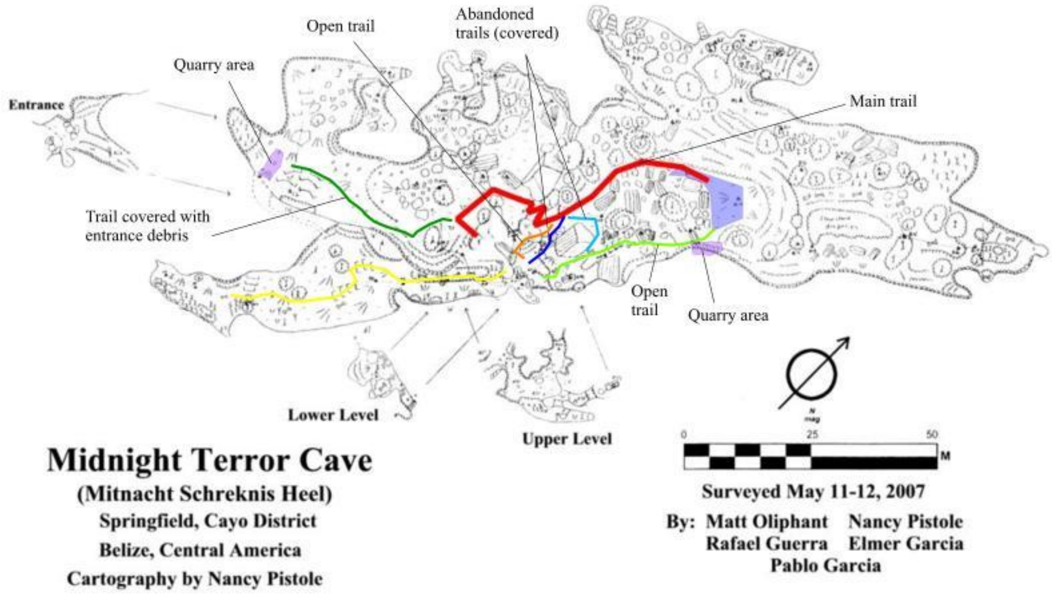

Figure 4. Ritual pathways through MTC.

Three seasons of investigation recovered large quantities of human remains and other artifacts. The largest assemblage was, not surprisingly, ceramics. Over 29,000 sherds were collected but the collection was by no means exhaustive. In a number of cases, the archaeologists discovered left the largely intact striated *ollas* in the cave so that they did not add to the project's storage problem. The heaviest concentration of ceramic was collected from Operation IV, particularly from around the edges of the large platform (Giron-Ábrego and Brady, 2014) (figure 5). Because excavations were not conducted, only a handful of sherds predate the extensive Early Classic modifications that buried earlier use surfaces. A moderately sized faunal assemblage (1,367 elements) was also recovered. Over half of the faunal material consisted of freshwater snail shells (*Pachychilus sp.*) known as *jute*, which have been shown to be part of Maya ritual cave assemblages (Halperin et al., 2003). The MTC assemblage differs from other cave assemblages in its relatively low percentage of mammalian bones and high percentage of fish (Orozco, 2019). Particularly interesting is the presence of four parrot fish (*Sparisoma viride*) that may have been brought to the cave as part of elite level sacrifices (Brady et al., 2019). The lithic assemblage features several chunky bifaces that appear to match tool marks left in clay banks in MTC where clay was removed for the construction of plazas and pathways (Figure 6).

Figure 5. Sherds of monochrone bowls and a striated olla (left) and polychrome (right) ceramic vessels.

Figure 6. A chunky biface (left) and corresponding tool marks on a clay bank.

1.2 The Human Skeletal Assemblage

MTC yielded over 10,000 human bones and bone fragments. Many more bones remain in the cave, cemented to the floor by calcium carbonate from cave drip water (Figure 7). All of the bone was collected from the surface. It is important to note that no excavations were undertaken except for a single, micro excavation in the side of a looters' pit to recover charcoal from on top of the clay floors exposed in the pit in Lot IV-07. In the course of the survey, the many looters' pits were checked but none exposed subsurface deposits of bone. In short, no evidence of buried human remains was found. The majority of the bone was recovered in Operations V and VIII, the lowest and highest chambers in the cave respectively.

Figure 7. Bones cemented to the floor.

The depositional pattern identified at MTC fits into a larger pattern of cave use in the Maya area (see Chapter 5). As noted in discussions of MTC (Brady and Kieffer, 2012; Prout and Brady, 2018; Verdugo et al., 2016), the MTC skeletal remains were recovered from the dark zone of a cave, highly fragmented, commingled and scattered across the floor (Figure 8). There a limited signs of violence on the bones and no discrete burials. Clear ritual use of the cave is evident through the use of a pathway along which are altars as well as terraces capable of holding numerous people. Not only does ceramic data show that this space was visited multiple times, but it was also visited over a long period of time.


Figure 8. Human skeletal remains scattered on the cave floor.

Subadult and adult remains were recovered in MTC. According to Prout and Brady (2018: 10), subadults comprise 25.7% of the total human skeletal assemblage. The authors also note that this figure is likely underestimated because the small size of subadult bones makes them more difficult to recover. Interesting, Prout and Brady's (2018) in-depth analysis of the subadult remains demonstrated that converse to a normal mortality curve, 42.2% of remains belonged to individuals between 5-10 years of age.

Other analyses of the MTC skeletal remains includes strontium isotope analysis by Lorenz (2017). In this study, strontium isotope ratios of tooth enamel from 30 subadults was assessed. Result show that 20 subadults were local to the

Belize River zone, three had geographical origin locations within the Belize Valley from along the Macal River, and seven had geographic origins outside the Belize Valley in the Central Maya Area.

My own research with the MTC collection began at California State University, Los Angeles in 2012 and continued into my doctoral program. Working with this material was challenging for a number of reasons. To begin, this is a highly fragmented, commingled, and scattered assemblage composed of thousands of bones. Bones remain cemented to the cave floor, to this day. Piecing together this material to generate data required patience and a great deal of time. Additionally, the cave is bereft of artifacts associated with the skeletal remains. What cultural material was recovered, was associated with religious ceremony and gave no information about these individuals. As such, this research has focused on obtaining demographic information of the individuals in the cave.

Bones have the ability to provide information on the sex, age, any trauma or pathology, and socio-economic status. This information, then, gives us the ability to identify patterns elucidating who these individuals were and why they were selected for "interment" in the cave rather than in rockshelters, another mortuary location used by the ancient Maya and contemporaneous with MTC. This information also allows us to re-examine hypotheses regarding the practice of human sacrifice by the ancient Maya which, as previously noted.

1.3 Research Questions

The large number of human remains, ritual cave context, and control of MTC by of Tipan Ch'en Uitz make it ideal for examining not only human sacrificial practices but also the social and political processes involved in cave use. Research questions for this dissertation include:

Question 1: Are individuals at MTC from the local settlement of Tipan Ch'en Uitz?
Question 2: Does the MTC population show preferential selection based on sex?
Question 3: Were men, woman, and children recruited from different populations?

The ability and extent to which each of these questions were addressed is discussed in the concluding chapter.

1.4 Methodologies

Research for this dissertation was conducted using osteological and paleogenomic techniques. Osteological analysis including estimating age, sex, any trauma on bone as well as determining MNI (see Chapters 2 and 4 in this dissertation). With this data, the population demographics of the MTC can be assessed for information on who sacrificed peoples were. A similar approach was also employed in the analyses of the skeletal assemblage recovered from the Cenote of Sacrifice (Anda Alanís, 2007). Using this data, I can answer question 2 which focuses on the sex differences in the MTC population and how they might relate to the practice of human sacrifice.

Paleogenomic analysis includes DNA extraction, creating libraries, and whole genome sequencing (see Chapter 2-4 in this dissertation). Questions 1 and 2 which analyzes the genetic relationship between MTC individuals and between MTC individuals and other Mesoamerican groups, can be addressed using the genomic data. Prevailing hypotheses suggests that a sacrificial population will be composed of non-local individuals. Genome-wide analyses of selected individuals provide information on the ancestry of individuals as well differentiate gene-flow, admixture, and direct migration / forced deportation patterns. If different amounts of genetic diversity are identified within varying sex and age range categories, this would reflect differing strategies of recruitment by sex and age.

In order to answer the research questions, it was necessary to combine osteological techniques with paleogenomic approaches because it allowed for a more holistic picture of the MTC sacrificial population. Because the remains are damaged, relying on only osteological analysis would have resulted in limited data. However, only examining the DNA would have left out other key data points such as age or signs of trauma. Each of these methodologies offered the ability to address population demography, both confirming and supplementing data provided by the other approach.

Detailed accounts of the methodologies can be found in chapters 3-5, in their respective "Materials and Methods" section.

1.5 Chapter Overview

Chapter 2 discusses the evidence for sacrifice at MTC. This outlines archaeological, ethnohistoric, and iconographic data, alongside the MTC skeletal remains to lay-out an argument for the practice of human sacrifice at MTC.

Chapter 3, published in the *International Journal of Osteoarchaeology* (Verdugo et al. 2016), counters arguments that social outcasts were sacrificed at MTC. In addition to refuting results of Kieffer (2015) based on our own osteological and paleogenetic analysis, the article also demonstrates that applying paleogenetic methods in challenging osteological and archaeological contexts can make results completer and more complex.

Chapter 4, published in the *Journal of Archaeological Science* (Verdugo et al. 2020), outlines the results of the analysis of dental modification in MTC. This paper discusses the hypotheses regarding dental modification practice by the Maya and the 102 modified incisors in the MTC collection. Paleogenomic data demonstrated that the practice is not sex-linked but may be used to distinguish lineages.

Chapter 5, published in the *International Journal of Osteoarchaeology*, outlines the analysis of the pelvic bones. Theoretical perspectives of human sacrifice are examined and discussed in relation to the MTC data. The high number of women present in the MTC collection suggests that women played a significant role in human sacrifice, possibly as deity impersonators.

Chapter 6 concludes the dissertation. This summarizes the overall findings as well as address the shortcomings of the data. Here, we also outline new questions and future research on MTC.

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CHAPTER 2

Addressing the Question of Sacrifice at Midnight Terror Cave

The recovery of human remains in cave spaces has prompted numerous discussions regarding the link between caves and skeletal remains since the start of the 20th century. Historically there is a pattern of disregarding the practice of human sacrifice as an explanation for human remains in caves. This may be related to the fact that habitation was considered the dominant function of caves for over a century (Mercer, 1896; Brady and Prufer, 2005). In this view, skeletal remains in caves was assumed to represent burial in much the same way as skeletal remains placed under house and patio floors. The problem was not resolved even when Thompson (1975) dismissed the idea of cave habitation because the rethinking all of the implications required another decade. Nevertheless, it is surprising that Thompson does not even discuss sacrifice considering the number of ethnohistoric references to child sacrifice in caves (Fuentes y Guzman, 1932: 33; Motolinía, 1971: 50; Scholes and Roys, 1938: 615; Tozzer, 1944: 44, n. 217; 180, n. 946; Zilbermann, 1966: 122). Additionally, Pendergast (1971: 18) interpreted a child's skeleton in Eduardo Quiroz Cave as a victim of sacrifice and Thompson had cited the report on other matters. The issue of sacrifice versus burial in caves was not explicitly raised in cave studies until the 1990s (Brady, 1989).

While the bulk of this research focuses on the osteological component of Midnight Terror Cave (MTC), the consideration of the larger question of human sacrifice has, necessarily, drawn from many more sources (Verdugo, 2018). At a minimum, the ancient Maya cave cultural context informed by ethnography and ethnohistory must be considered. Equally important is the consideration of archaeological context. However, the limitation of all these sources of data will also be considered.

2.1 Caves as Non-Normal Burial Spaces

In the interpretation of the MTC skeletal assemblage, it should be noted that early in the 20th century, Oliver Ricketson (1925: 392–394), concluded that caves were not a usual burial place. Ricketson states, "But on the whole, the evidence is strongly against the use of caves in Yucatán as a usual burial place. Had it been so, the dense population which must have existed when the Maya were at their height, would probably have rendered these caves archaeological treasure houses" (Ricketson, 1925: 394). More recently, Welsh (1988: 3) excludes cave burials from consideration in his analysis of burial practices saying that "burials may have had a different role and purpose from site burials and should therefore be considered separately". This is an important point because, if caves are not a usual burial place, it is just as incumbent on the side arguing for burial to justify that interpretation as it is for the side arguing for sacrifice.

If caves are not normal burial places and if the skeletal remains appear to have a "different role and purpose," it is important to determine the nature of that space. This begins with acknowledging that the significance of caves in Maya thought is quite different than in Western thought. As such, any interpretation of activities occurring within caves must be contextualized within a Mesoamerican perspective. In

Maya cave studies, the definition of "cave" is derived from the Maya term *ch'e'en* which denotes a cave but is also applied to a number of other features that penetrate the earth (Verdugo et al., 2016). For example, the name of the site of Chichen Itza is generally translated as "at the edge of the well of the Itza" and refers to the Sacred Cenote. The form, Chi-*chen* Itza, clearly shows that the Maya considered the cenote to be a cave. Thus, MTC shares the same *emic* category with the Sacred Cenote.

The differences between the Maya and the Western perspectives do not end there. While rain is a celestial phenomenon in Western thought, it is a terrestrial one in Maya culture (Vogt, 1969: 387). Numerous ethnographic studies document the Maya and Mesoamerican belief that rain is formed in the earth and it is for this reason that petitions for rain are often made in caves (Early, 2006: 64-65; Huicochea, 1997; Morris, 1986: 57; Sepulveda, 1973). This is relevant to the current discussion because Bishop Landa noted in the 16th century that human sacrifice is most often carried out as part of rain petitions (Tozzer, 1944: 180, n. 948). Given the cave's primary function as a place of ritual and its association with rain, sacrifice becomes a likely option.

The close connection to rain is obvious in cave sites. Brady (1989) was the first to point out that activity tends to cluster in wet areas of the cave. If most human sacrifice is related to rain petitions, one would expect these rituals to occur in wet areas (Prout and Brady, 2018). Ethnohistoric sources confirm this suggestion. The 17th century historian, Francisco Antonio de Fuentes y Guzman (1932: 336), reports that children were sacrificed to bring rain in a cave near the site of Mixco Viejo that contains a spring called the "mother of water." Archaeologically, this was demonstrated at Petroglyph Cave, Belize, where subadult skeletons were found cemented in pools behind rimstone dams (Reents-Budet and MacLeod, 1986). Even outside of caves, watery areas were important for sacrifice as shown by the Aztec sacrifice of children in the lake (Sahagún, 1981: 43). At the dedication of Tenochtitlan's aqueduct, a child was sacrificed at every outlet (Duran, 1964: 211). This point is of particular interest because wet areas are not chosen as places of burial. Human skeletal remains recovered from wet areas, then, is likely related to sacrifice, not burial (Owen, 2005).

In reviewing the more than a century of cave exploration in the Maya area, the presence of human skeletal remains are an element that has consistently caught the attention of explorers and is commonly reported (see Brady, 1989: 343-344 for a listing of more than 50 instances). The information provided is often frustratingly incomplete with basic details such as the number of individuals present or body position usually lacking. An obvious, significant aspect of the ritual is the exposure of skeletal material on the cave floor rather than burial. This point will be examined in greater detail in the discussion of MTC, but it should be recognized that MTC fits within this larger pattern.

It should be noted, however, that examples of deliberate interment have also been recognized. Tomb burial, cyst burial, and interment within sealed alcoves have been documented in Maya caves (Brady, 1989; Garza et al., 2001; Kieffer, 2009; Lee and Hayden, 1988). Thus, there are clear contextual and depositional differences between recognized burials and the pattern of surface remains.

To summarize, this section has provided some general background on caves and the nature of skeletal assemblages recovered from them. Caves are not normal burial places with examples of cave burial being rare. Nevertheless, well documented examples exist. Skeletal remains are most often found scattered and comingled on the surface. Caves are associated with ritual and are believed to be places where rain clouds are formed. For these reasons, caves are more likely associated with sacrifice than burial and caves share the same conceptual category with the Cenote of Sacrifice.

2.2 Burial: A Consideration of Cave Ossuaries

In investigating whether MTC reflects burial or sacrifice, it is important to first considered what form of burial practice might account for 10,000 comingled bones scattered across the cave floor. This is necessary because ultimately the discussion must come down to two alternatives and our analysis will determine which more closely fits the MTC data.

The most likely burial option to consider involves some form of cave ossuary. The term “ossuary” has been applied very uncritically in the past to refer to any place where the skeletons of multiple individuals are found (Jirikowic, 1991). For this discussion, the term refers to a multistage mortuary practice where deceased persons undergo primary interment, followed by a collective secondary burial in a formal place of deliberate interment (Ubelaker, 1974; Jirikowic, 1991). This can range from simple re-burial or storage. Often "there is characteristically little or no attempt to keep the remains of particular individuals or groups of individuals separate from those of others within the secondary burial" (Jirikowic, 1991; 355).

The question, then, is: Do cave ossuaries exist in the Maya area and, if so, what do they look like? The best-known example has been Gordon's Cave #3 at Copan, Honduras and is described as:

> The walls are black, the air close and foul, and altogether it is as repulsive a hole as could be found in the face of nature. The floor seemed more uneven than in either of the other chambers, and gave way still more to the pressure of the feet, and with a crushing sound. I soon discovered that I was walking upon the dust and crumbling bones of decomposed human bodies, mingled with ashes and lime. A mass of charred and calcined bones occupied the entire floor to a depth of about two feet (Gordon, 1898: 143).

Although located a good distance from the Maya lowlands, Copan has always been considered a lowland site because of its art and architecture. The site took on the lowland affiliation when elites from Tikal established a dynasty at Copan in the 5th century A.D. A restudy of Gordon's Cave #3 in 1983 estimated Chamber 3 to contain 600-700 individuals (Rue et al., 1989). One of the interesting features of Gordon's original study was the collection of a number of ceramic vessels against the inner wall of the chamber which were recognized as being earlier than any ceramic from the site (Gatschet, 1898; Thomas, 1898). The ceramic vessels were later identified as being at least Middle Preclassic [800 – 400 B.C.] in date (Porter, 1953: 54) and so predate the intrusion of lowland Maya culture by more than a millennium.

Although Chamber 3 is described simply as holding a mass of bone, Gordon does note a seated, flexed adult buried under an overhang in the cave wall in Chamber

1 that crumbled into dust on being touched (Gordon, 1898:143). The skeleton was accompanied by a ceramic vessel. Rue and colleagues (1989: 398) also recovered the remains of an infant less than two years of age wrapped in a cloth shroud in Chamber 1. During an additional restudy in 1991, Brady (1995) uncovered the remains of several individuals in Chamber 1. One of these was associated with the remains of a cloth bag suggesting that this was a secondary burial brought to the cave in the bag. Another was accompanied by the remains of basket while a third was overlain by the skull of a child and accompanied by a leather bag that appeared to hold the skull of another child. While deterioration over three millennia as well as cremation of adult remains had blurred evidence of discrete individuals in Chamber 3, the identification of individuals was noted in the first chamber by all three projects.

The interpretation of the Gordon's Cave ossuary profoundly changed with the discovery of a series of ossuary caves in Olancho in eastern Honduras. These were the first ossuaries to be radiocarbon dated and placed their utilization between 1400 – 850 B.C. This fits well with the ceramic dating from Copan and the Cuyamel caves (Healy, 1974). The discoveries focused attention on Honduras where Doris Stone recorded a number of ossuary caves, identifying them as a Honduran burial pattern (Stone, 1957: 56). The widespread distribution in Honduras and corresponding absence in eastern Guatemala along with their early date, eliminated Gordon's Cave from consideration as a Maya burial type (Scott and Brady, 2005: 272).

The best investigated of the Olancho ossuaries, the Cueva de Talgua, provided substantial information on caves used as ossuaries. A common depositional pattern had long bones laid neatly side by side with smaller bones piled on top and the skull at the apex. This left little doubt that these were secondary burials. In several places, a large number of skeletons had been placed in close proximity. Any evidence of discrete boundaries and connections to ceramic and marble vessels, jade and shell beads, had been erased by subsequent deterioration of the bone. Still, other remains showed clear connections between the individuals and mortuary offerings. While these ossuaries are not Maya, it is helpful to see discernable individual remains and the presence of mortuary offerings. At the very least, it is instructive to note how different the depositional pattern in these ossuaries is compared to MTC where, by contrast, no attempt was made to preserve the integrity of individual bodies and no mortuary offerings were found.

A number of ossuaries were also reported from Chiapas, Mexico by Frans Blom. Blom states:

> The cave of Huxjal is quite large and several narrow crevices branch off in many directions. One of these crevices was filled with human bones and a skull appearing to hold a longbone in its mouth was staring at us as we entered (fig. 1). Bones and skulls were piled up helter skelter and the one thing which was quite plain was that all the skulled showed artificial deformation, making it clear that we stood before an ossuary of Maya origin (Blom, 1954: 123-124).

Unfortunately, no additional details are given making any type of interpretation risky.

Moxviquil Cave had a number of individuals lying on the surface under dripping water so that the bones were cemented together. Although these caves resemble MTC, so few details are given in the description that they are impossible to interpret. Some details, like the presence of skeletal remains laying on the surface, raise questions about whether these were actually burials. It appears that any cave containing multiple individuals was labeled an ossuary by Blom (Scott and Brady, 2005: 272).

Blom also mentions another type of ossuary involving cremation. Although not in a cave, Blom (1954: 124) found an ossuary in a subterranean cruciform masonry chamber at Tzajalob, Chiapas. The chamber was filled with ceramic jars containing cremated human remains. At least one of these vessels contained a small jade head. This feature was similar to Cieneguilla Cave, a small walled up cave that had contained urn burials (Blom, 1954:126). The ceramics and the textiles date to the Postclassic (O'Neale, 1942; Wauchope, 1942). Chiptic Cave and Rosario Trabajo are also ossuaries similar to Cieneguilla Cave with Chiptic Cave producing a copper bell in one of the urns (Blom, 1954: 127).

Blom also identified the caves around Lake Lacandon as containing ossuaries. Here, bones were piled up on the surface near the entrances as well as found deeper in the caves in crevasses. Blom also reports a large block of bone cemented together by dripping water. The caves around Lake Lacandon appear to be somewhat different than the other caves described by Blom. Scott and Brady (2005: 273) hold out the possibility that the Cave of Mensäbäk represents an ossuary based on Gertrude Dube's visit in 1943, in which she notes the presence of the skeletal remains but does not mention evidence of looting (Blom and Duby, 1955: 350). McGee (1990: 59) notes that all the skulls at the Cave of Mensäbäk show modification. Petryshyn (1969), in contrast, noted only that some of the skulls at the nearby Cave of Tsibaná were not modified. This is an important point because the presence of unmodified skulls suggests that skeletal remains continued to be added into the historical period. While Blom (1954: 132) says that "the skulls and bones had been placed without specific plan," McGee (1990: 58) notes that four skulls were apparently recently arranged on the ground with god pots around them. This indicates continued manipulation of skeletal elements in rituals by the Lacandon. Cucina and Tiesler (2014: 248) document this occurring and correctly call the taphonomic histories 'overwhelming'. The assessment is that these caves may have been ossuaries but that the information currently available does not permit us to form a clear impression of what was occurring.

Cucina and Tiesler (2014) have provided a clearer picture of another feature around Lake Lacandon. According to Cucina and Tiesler (2014: 248), "Only a single test pit has been excavated in one of the rockshelters, which revealed a heavily disturbed primary seated burial, confirming that the surface deposits are ossuaries, of commingled, selected bony segments, rather than primary surface burials". What is significant, however, is Cucina and Tiesler's identification of the feature as a rockshelter. Based on the work of Juan Luis Bonor (1995; Bonor and Martínez, Klemm, 1995; Glassman and Bonor Villarejo, 2005) at Caves Branch Rock Shelter, Belize and Keith Prufer's (1997; 2002; Saul, Prufer, and Saul, 2005) work on the Maya Mountains Archaeological Project, Scott and Brady (2005: 273) identify rockshelters as a particular form of cemetery/ossuary. Subsequent work by Wrobel and colleagues (2007, 2009) at Caves Branch Rockshelter has further elaborated this.

As the cemeteries filled up, new graves often cut through older burials and these unarticulated bones were backfilled on the newer burial. Thus, varied depositional contexts may be identified but it does not alter the basic pattern of individual interment. Rockshelter cemetery/ossuaries contain individual burials, some of which contain mortuary offerings so, once again the pattern is distinctly different than MTC.

Finally, J. Eric Thompson (1975: xxxiii) recounts a 17th century incident in which Bishop Francisco Núñez de la Vega removed and burned bones from a cave in Chiapas that were being worshipped as the group's founder. Thompson identified this activity as a lineage cult reflecting ancestor worship. According Swanson (1960), ancestor worship tends to be found in societies where kin groups are important decision-making bodies which fits Chiapas. In this same area, ethnographer Alfonso Villa Rojas (1969: 215) was told that in previous generations, certain prominent old men were buried in the lineage cave, indicating the existence of cave ossuaries. Understandably, archaeological evidence verifying the existence of a lineage cave ossuary has not been produced because archaeologists would not be permitted to investigate such sites by the local Maya. Even here, however, we do not have a good match for MTC. The cremated bodies maintained their separate identities by being placed in separate urns (Villa Rojas, 1969: 215). Furthermore, the lineage and clan organization, while strong in the Western Highlands, quickly becomes attenuated as one moves east so one has to wonder if kin groups were important enough to generate ancestor worship at MTC.

In summary, the term ossuary has been used very loosely to refer to caves that contain the remains of multiple individuals. A number of these resemble MTC in having skeletal remains exposed of the surface. However, the lack of information provided does not allow us to confirm that these are formal ossuaries. Those accounts mirror the situation mentioned at the beginning of the paper for cave skeletal remains, in general. Since interment in caves has been recorded in tombs, sealed alcoves, and cysts and cemeteries of interred individuals have been found in rockshelters, it appears doubtful that scattered remains on the surface represent burial. Although Scott and Brady (2005: 272) conclude that ossuaries are not well documented, several examples of formal ossuaries are noted in the Chiapas highlands. The caves appear to be associated kin groups such as linages or clans (Guiteras Holmes, 1952: 103). The ossuaries feature cremations with the remains placed in ceramic vessels. In some cases, valuables associated with the deceased were included in the vessels. All of the ossuaries show a concern for maintaining the integrity of the remains as separate individuals and so are quite different than the scattered and comingled remains at MTC. The accompanying offerings also have no counterpart at MTC. The evidence argues persuasively that MTC is not an ossuary.

2.3 Identifying Sacrifice

Given that MTC does not match the pattern for ossuary use of cave space, attention turned to the other prevailing hypothesis for cave use; the practice of human sacrifice within cave space.

According the Buikstra (2007), academic interest in sacrifice has only developed since the 1960s. With recent examination and re-examination of sacrificial assemblages (Anda Alanís, 2007; Beck and Sievert, 2005; Lucero and Gibbs, 2007), discussions have focused on identifying patterns or processes associated with human sacrifice still visible on the skeletal remains or, within the archaeological context (Berryman, 2007; Tiesler, 2007; Scott and Brady, 2005). This is particularly challenging in the Maya area with the generally poor preservation of skeletal remains. Because of this, many sacrificial discussions have relied on documentary or contextual data, often ignoring biographic or taphonomic evidence (Tiesler, 2007: 16).

Focusing on depositional and funerary conduct, Tiesler (2007: 22) distinguishes expected taphonomic patterns between funerary and sacrificial deposits through the examination of the biological profile, form of death, pre-depositional body treatment, primary deposition, post-depositional manipulation, and secondary deposition. Tiesler's (2007) methodological approach, combining archaeology, iconographic, and ethnohistoric data with taphonomic signatures, is the model for the systematic approach in this analysis of MTC skeletal assemblage.

Berryman's (2007: 394) approach is particularly useful as it outlines five depositional characteristics found in sacrificial assemblages which include:

> 1) placement of bodies in highly visible public or ceremonial spaces (in contrast to the typical residential mortal patterns for the region, apart from high-status people);
> 2) lack of investment in grave preparation;
> 3) presence of a clear selection for certain members of the population;

4) lack of mortuary offerings; and
5) signs of violence (dismemberment, cutmarks, etc.).

Many of these categories mirror Tiesler's (2007) for post-sacrificial deposits. With the archaeological recovery of skeletal remains being an early step in bioarchaeological analysis, Berryman offers useful points for defining sacrificial contexts. The MTC skeletal assemblage matches a number of the previously outlined features identified by Berryman (2007) and Tiesler (2007) as pertaining to post-sacrificial deposits.

Berryman's first point, the placement of bodies in highly visible or ceremonial spaces, is exemplified throughout MTC. For example, Operation V, the deepest and wettest part of MTC, has a number of large terraces that could have accommodated several hundred people. Brady and Kieffer (2012: 251) argue that this space is associated with the public demonstration of sacrifice. MTC, then, also reflects Inomata's (2006) ideas of performance and power, making it an ideal location for assessing the role of human sacrifice as a social and political phenomenon. This was particularly apparent in the lower portion of Operation V where a piece of ceiling collapse had been converted an altar. The sacrifices appear to have been performed on the altar and bodies were simply rolled off the altar and down a slope where they decomposed. The area also illustrates a point made at the beginning of the chapter about the use of wet spaces. Most of the bone had been cemented to the floor by calcium carbonate from dripping water so these bones were never collected as part of the MTC assemblage.

Berryman's second point, lack of investment in interment, is related to her first point, and often noted to be indicative of sacrifice (Brady, 1989; Brady and Stone, 1986; Gibbs, 2000; Lucero and Gibbs, 2007). At MTC, this is demonstrated in the surface discard of bodies that allowed skeletal remains to become commingled and scattered across the cave floor. Along the ritual circuit going through the cave, bone may have been tread on or kicked to the side by traffic. A ritual platform constructed around a speleothem column in the upper portion of Operation V provides a telling story of post sacrifice body treatment. The platform had hundreds of smaller bones, such as phalanges, imbedded in its surface. The larger bone had apparently been gathered up and deposited in a seasonal pool at the lowest point in the chamber. The removal of the remains of previous ceremonies before performance of the next ritual is a pattern among modern Maya ritual specialists (Scott, 2009) and mentioned archaeologically at several sites (Brady, 1989; MacLeod and Puleston, 1979). The deposition of the remains in the intermittent pool reinforced the point made early in the chapter concerning the importance of watery spaces in sacrifice. Secondly, the apparent lack of concern with maintaining the integrity of the body is striking. Not only was the bone comingled in Operation V, the removal of parts from the upper platform separated body parts. This contrasts markedly with the concern noted in the discussion of formal ossuaries of maintaining individual integrity.

Surface deposition of human remains is the most frequently encountered characteristic of cave skeletal remains and is significant as one of the most obvious indicators of sacrifice. Still, the point~~ed~~ has been contested in more recent cave studies. Wrobel and colleagues (2014: 77) state of the skeletal remains found on a wet, muddy cave floor at Je'reftheel, "provide a strong analogy to funerary behavior documented in tombs throughout the Maya region and beyond." The analogy between comingled cave remains and comingled tomb remains has already been criticized for its inappropriateness (Prout and Brady, 2018: 7). It is important to stress is that there is a clear difference between remains left on the surface, in plain view of visitors and remains that were sealed or covered. Thus, there is strong analogy between surface deposition and tomb burial only if one choses to ignore the fact that former exhibits two of the principal characteristics of sacrifice while the later does not. As has been noted earlier, actual cave burials have been found in tombs, sealed alcoves, or cysts, so, if these are supposed to be elite burials, why are they not in such structures?

It is ironic that the first application of paleogenomics with the MTC assemblage (Verdugo et al., 2017), was aimed at disproving a proposed example of Berryman's third point, "presence of a clear selection for certain members of the population." Studies (see this dissertation) clearly showed that Kieffer's (2015) suggestion that victims may have been selected for sacrifice based on having a physical deformity could not be sustained. Additionally, Scott (2016) underscored the importance of embedding any interpretation within the appropriate cultural context.

Subsequent work on the MTC assemblage suggests the selection of certain members of the group. Analysis demonstrates that 43% of the MTC individuals are subadults and that the greatest number died at ages when mortality is generally low

(Prout and Brady, 2018). This pattern, like the mortality curve of the Cenote of Sacrifice at Chichen Itza (Anda Alanís, 2007), differs from a normal Mesoamerican mortality curve identified by Storey (1985) in her study of a residential unit at Teotihuacan. The MTC subadults align with ethnohistoric data identifying children of five to six years of age as most frequently selected for sacrifice (Prout and Brady, 2018). Additionally, the MTC results are congruent with a larger pattern documented by Cucina and Tiesler (2014: 243) which identifies infant and subadult remains found "in caves, cenotes, and other natural underground places … as clearly distinct from the natural mortality profile, denoting in some cases a cultural pattern involved in the preferential deposition of individuals in this age range".

Our analysis of the pelvic bones from ones MTC also demonstrates the selection of specific member of the population. Examination of the pelvic remains uncovered that adult females are either very young adults or in the middle age range (Verdugo et al., 2020). These two age ranges are particularly interesting because they correspond to a widely recognized division of female deities in Mesoamerica into a young and an old aspect (Thompson, 1939) and has been noted in both ethnohistory and iconography. In the Maya area, numerous accounts mention the sacrifice of women who fall into the two age ranges we have identified (See Verdugo et al., 2020 for full discussion).

Berryman's fourth point that the sacrificed deposits "lack of mortuary offerings" is perhaps the most widely accepted (Brady, 1989: 351; Fowler, 1984; Gibbs, 2000; 2007: 67; Kieffer, 2009; Owen, 2005; Peterson, 2006). It needs to be clear, however, that while the presence of mortuary offers argues persuasively for burial the lack mortuary goods does not make the same argument for sacrifice for the simple reason that interments often lack offerings. At MTC the contrast in artifact assemblages is stark. The densest concentration of ceramic is in the front chamber of the cave where there is little or no human bone. Correspondingly, Operation V and VIII that hold the majority of the skeletal remains have little ceramic. This suggests that very different types of ritual were performed in the different areas of MTC (Prout and Brady, 2018; Brady and Kieffer, 2012). In short, there is nothing at MTC that appears to be a burial offering and with the number of individuals present, this is significant.

One must be cautious, however, in describing artifacts as burial offerings. Wrobel and colleagues (2014: 95) propose that two bifaces from Je'reftheel cave with no use wear as perhaps having been manufactured for inclusion in an interment. It is just as likely, however, that the lack of use wear may be attributable to a very short use life. Furthermore, neither blade was described as being found in close association with skeletal remains. Finally, chert lanceolate bifaces are a common cave artifact. Seventy-two were found in five different caves in the Petexbatun area and in 16 other caves (Brady, 2005: 127). In 1957, Holland and Weitlaner (1960) found three blades hafted in Pre-Columbian handles stored in a cave in the Cuicatec area of Oaxaca. The blades were still in use as sacrificial knives by ritual specialists in the village. Clearly the significance of these artifacts needs to be rethought.

Berryman's (2007) last point describes signs of violence as the final characteristic of sacrificial deposits. While signs of violence appear in the MTC skeletal assemblage, they are infrequent. This may be due to the buildup of calcium carbonate on the bone from dripping water. Buildup has obscured more subtle bone features and left many remains cemented to the cave floor. Still, Kieffer (2015) identifies 28 cases of perimortem trauma in the collection, including cutmarks.

"Signs of violence" as a marker for sacrifice needs to be examined in more detail. It is interesting that in the first analysis of a sacrificial assemblage, Hooten's (1940) report on the bones from the Cenote of Sacrifice, evidence of violence was not even noted. Nevertheless, this particular pattern appears to carry more weight than others in assessing sacrificial assemblages perhaps because it is the one piece of evidence directly tied to the bone. Many would agree with Pendergast's (1971) assertion that, without a clear indication of violent death, an individual cannot be interpreted as a victim of sacrifice. This, however, is an oversimplification of a complex problem.

Scott and Brady (2005) argue this point for two reasons. First, ethnohistoric literature records various manners of sacrifice that leave no obvious signs on human remains including strangulation, suffocation, disembowelment, drowning or imprisonment in caves (Tozzer, 1941; Scott and Brady, 2005). This points to the ritual killing of people beyond the more popular heart excision seen with Aztec practices. Even heart excision has been the subject of academic reexamination with several authors suggesting that incisions were made through the diaphragm rather than breaking through the rib cage (Robiscek and Hales, 1984; Tiesler, 2006). This method would leave fewer signs of violence and in different locations than generally associated with heart excision. Finally, the practice of assuming individuals died of natural causes when no evidence suggests the contrary is only applicable to normal burial contexts of which MTC is clearly not (Scott and Brady, 2005). The assumption, then, that sacrifice can only be identified as such when trauma is present is problematic because it assumes that all forms of sacrifice leave recoverable signatures identifiable on human remains.

2.4 Conclusions

This section has reviewed the evidence for both burial and sacrifice at Midnight Terror Cave with a general discussion of caves. It noted several authors commenting that caves are not a typical burial place which served as a warning against simply assuming burial. It then discussed caves as ritual space and noted that the Cenote of Sacrifice at Chichen Itza is explicitly designated as a cave by the Maya. It went on to point out the close connection between caves and rain in Maya thought which is important because Tozzer says that most sacrifice occurs in the context of petitions for rain. The archaeological utilization of caves also noted a close connection with wet areas and dripping water and points out that skeletal remains deposited in wet areas is more suggestive of sacrifice than burial. While the cave literature tends to be lacking in useful details about skeletal remains in caves, it is nevertheless clear that it is almost always referring to bones deposited on the surface which is also the case at MTC. In contrast, the few cases of actual burial are found in tombs, sealed alcoves, and cysts within the cave system. Thus, the general discussion of caves makes sacrifice appear to be the more likely than burial as an explanation for the presence of human skeletal remains.

The investigation of the possibility of burial began with the premise that for an assemblage of 10,000 bones to represent burial, the context must be an ossuary. Unfortunately, this term has been used uncritically in the past and so is restricted here to a formal, deliberate place of interment used by some segment of society. In agreement with Scott and Brady (2005), Maya cave ossuaries are rare. Gordon's Cave #3 at Copan was eliminated because it is not Maya but belongs to an Early/Middle Preclassic Honduran pattern of burial. Cave ossuaries were noted but limited to the western highlands where lineage organization is strong. Ossuaries may range as far as Lake Lacandon in the lowlands, but this is uncertain because of the "overwhelming" character of the taphonomic histories of these caves. The existence of rockshelter ossuaries is well recognized but these differ markedly from deposits in deep, dark zone caves. All ossuaries differ from MTC in showing concern for maintaining the integrity of the individual bodies interred and shielding them from public view either through burial (rockshelters) or the sealing of cave entrances after deposition. MTC is clearly not an ossuary.

On the other hand, MTC shows characteristics of sacrifice noted by Berryman (2007), Fowler (1984), Tiesler (2007) and others. The "lack of investment in grave preparation" is clear in the scattered and comingled surface deposition as is the "placement of bodies in highly visible public or ceremonial spaces" and the "lack of mortuary offerings.” The “clear selection for certain members of the population” has been demonstrated for subadults (Prout and Brady, 2018). Finally, “signs of violence,” while not numerous, have been noted (Kieffer, 2015). In weighing the lack of fit with burial and close correspondence with the characteristics of sacrifice, it is difficult to escape the conclusion that MTC represents a sacrificial assemblage.

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CHAPTER 3

Ancient DNA Clarifies Osteological Analyses of Commingled Remains from Midnight Terror Cave, Belize

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Authors: Cristina Verdugo[1,2], Kalina Kassadjikova[2], Eden Washburn[2], Kelly Harkins[2], and Lars Fehren-Schmitz[2]

Key words: Paleogenetics, Klippel-Feil Syndrome, Maya, Midnight Terror Cave, Haplotype

Abstract:

In the past two decades, paleogenetics has made a significant impact on the field of archaeology. Interestingly, paleogenetic methods have not been extensively employed in osteoarchaeology despite its ability to address issues that cannot be resolved through traditional osteological analysis alone. This paper tackles a problem concerning the relationship between human remains in a comingled deposit from Midnight Terror Cave (MTC), Belize. Paleogenetics demonstrates that the bones in question belonged to multiple individuals, thus resolving an issue that was at the heart of the interpretation of social processes at the site.

[1] All correspondence and proofs should be sent to: Cristina Verdugo, Department of Anthropology, University of California, Santa Cruz, 1156 High St, Santa Cruz, CA 95064, USA. Telephone: (714) 403-3887. Email: cverdugo@ucsc.edu. [2] University of California, Santa Cruz, 1156 High St, Santa Cruz, CA 95064, USA

3.0 Introduction

Midnight Terror Cave (MTC), located in the Cayo District of Belize, has produced an osteological assemblage of over 10,000 bones, most of which belong to victims of human sacrifice. Considering the dearth of large sacrificial assemblages, MTC holds promise for clarifying our understanding of ancient Maya human sacrifice. In dealing with human sacrifice, osteological analyses, under the best of conditions, provide insight into general health, age, sex, skeletal pathology, and evidence of physical violence suffered by an individual. The contribution of osteological analysis, however, is heavily dependent on the preservation of the archaeological context and the skeletal remains themselves. Where skeletal material is mixed and comingled, osteological analyses are more limited in what they can say. Preservation of human bone in the Maya area is generally poor as a result of the moist, humid climate and acidic soil. In such contexts, attempts to use osteological analyses to address larger issues of social process can be fraught with difficulties.

3.1 Paleogenetic Analyses in Osteoarchaeological Contexts

Paleogenetics, while also constrained by problems of bone preservation, has the capacity to augment traditional osteoarchaeological analyses. The field of paleogenetics has increasingly been applied within archaeological contexts over the last two decades to reveal population dynamics, social processes, and in reconstructing phylogeny and evolutionary history. While ancient DNA (aDNA) analyses have contributed greatly to these broader categories of the human past, paleogenetics has been infrequently applied to individuals in ways that contribute to osteoarchaeological analysis. For example, under certain conditions, aDNA analysis can estimate biological sex more accurately than osteological sexing methods which are susceptible to inter and intra-observer error (Kaestle and Horsburgh, 2002; Skoglund et al., 2013). Given that the majority of osteological markers used for sex estimation do not appear until the adolescent stage of life, skeletal remains of subadults are particularly difficult to sex accurately using osteological markers and morphometric measurements alone. In these cases, sex is often left undetermined or, at best, an educated guess (Faerman et al., 1998; Kaestle and Horsburgh, 2002; Lassen et al., 2000; Schutkowski, 1993). Accompanying grave goods may provide insight to gender, however, many scholars have shown that this assumption can be highly problematic and prone to Western bias on the part of the investigator (Gellar 2008).

Cruz and colleagues (2008), for instance, employ aDNA testing to determine the sex of 37 subadults and six adults recovered from Temple R at the site of Tlatelolco in Mexico City, a ritual context that coincides with ethnohistoric descriptions of sacrifice to the Tlaloque, the Aztec rain dwarfs. Results from Temple R illustrated the successful amplification of DNA from 32 of the 37 subadults as well as from all 6 adults, however, the results could only be replicated in 26 of the individuals. Cruz and colleagues (2008) obtained a 60% success rate of sex identification using aDNA extraction. The data lend support to ethnohistoric evidence indicating the preference for male victims who could better personify male deities. Results here demonstrate the ability of aDNA studies to provide support for archaeological interpretations. In this context, identification of sex became extremely important in corroborating ethnohistoric accounts that relate the sacrifice of same sex deity impersonators to rites directed to specific deities.

Finally, the ability for aDNA analyses to address issues regarding genetic disease also plays a key role in reconstructing the lives of individuals in the past. Recent studies have explored mutations associated with particular congenital diseases or genetically determined disease susceptibilities as well as focused on sequencing genomes of ancient pathogens causal for infectious diseases (e.g. Bos et al., 2014; Lin et al., 2015; Mathieson et al., 2015).

This paper reports on a case from Midnight Terror Cave, Belize in which paleogenetic analysis clarifies and supplements osteological data as well as resolves an osteoarchaeological problem.

3.2 Sacrifice at Midnight Terror Cave

Recently, C.L. Kieffer (2015a) reported two sets of fused vertebrae (VI-02B-118, VIII-13-367) from MTC that she identified as possibly suffering from Klippel-Feil Syndrome (KFS) and suggests that the Maya may have chosen the two individuals for sacrifice due to resulting deformities. Kieffer (2015a) contends that these cases may reflect a larger social phenomenon in which the victims of ancient Maya human sacrifice were selected on the basis of being considered "social outcasts."

Kieffer's (2015a) position was almost immediately questioned. After examining both vertebrae, Prout (2015a) contends that one specimen (VI-02B-118) was misidentified and instead asserts that the bone displays an abnormal growth development of the C-2 and C-3 vertebrae. Prout (2015a) notes that with only the C-2/C-3 vertebrae present in Lot VI-02, there is no evidence to claim a condition more serious than type II KFS and quotes Barnes (1994: 69) as saying, “Type II defects are the most commonly occurring block vertebrae, and they generally produce no symptoms.” If individuals were generally asymptomatic with type II KFS, it is unlikely that they would have been selected for sacrifice based on this condition.

Kieffer (2015b), however, argues that other pathological adult bones in Lot VI-02, when taken together with the vertebrae, manifest abnormalities consistent with type I KFS. These conditions include evidence of spina bifida occulta in the sacrum, signs of porotic hyperostosis on the frontal, parietal and occipital bones, osteoarthritis on a thoracic vertebra, and possibly supernumerary incisors. At this point, the discussion stalled because traditional osteoarchaeological analysis does not have the ability to determine if particular bones belong to the same individual when recovered from co-mingled contexts. This is precisely the type of question that can be addressed by paleogenetics. As Scott (2015) underscores in describing this issue, if the bones did belong to a single individual and that person did suffer from type I KFS, they would undoubtedly have been treated as a disabled or deformed person. Scott shows that such individuals were not socially marginal but rather held valued and socially important statuses. The disabled and deformed appear to have been rarely sacrificed but when it did occur, it had potentially important social implications.

Before presenting our paleogenetic data, it should be noted that the context of this find as laid out in "Sacrifice of the Social Outcasts" is more problematic than acknowledged. Lot VI-02 was reported as having a minimum number of individuals (MNI) of two, an adult and a juvenile, so it would, at least theoretically, be possible to assign all of the bones to one individual or the other based on size and development (Kieffer 2015a). In reality, however, it is well recognized that an MNI of one does not mean that all the bones belong to a single individual. During our analysis of Lot VI-02B, the presence of at least two adults was documented based on the recovery of two fairly complete occipital bones, two left calcanei, two right first metatarsals, two right fourth metatarsals, two right ischia, and two fragments of maxillae.

Similarly, Prout's (2015b) analysis of the juvenile remains from Lot VI-02B documented four individuals. In addition to the juvenile mentioned by Kieffer (2015a), there is a child of approximately 2-4 years of age, a subadult 6-10 years of age, and another subadult 10-14 years of age.

The archaeological context is significant here. Most osteological deposits at MTC show scattering and comingling. Field notes for Lot VI-02 record a number of looter's pits along with piles of rubble from those pits and ceramics stacked by looters. It should also be noted that Lot VI-02 is not a spatially discrete lot but was arbitrarily separated from VI-01 to provide some spatial control over a long section of passage. Lot VI-02 is mixed and comingled with no fewer than a half dozen individuals and likely intermixed with Lot VI-01. Given the complete context, the assignment of bones with various pathologies to the single individual with the fused vertebrae is untenable by traditional osteoarchaeological standards. Our paleogenetic analysis, however, is unaffected by this added complexity and so is applicable to a wide range of contexts.

3.3 Paleogenetic Analysis

The suggestion raised by Kieffer (2015a) of the bones' relation to each other, however, persists as a valid issue. The chance that all the bones belong to a single individual remains a possibility, which, until now, has been unresolvable through traditional osteoarchaeological techniques. We attempted to establish the relationships between bones in Lot VI-02 more precisely by subjecting MTC bone samples to paleogenetic testing in the Human Paleogenomic Laboratory at the University of California, Santa Cruz. Twelve samples from Lot VI-02 included: the vertebrae thought to show KFS (VI-02B-118), the sacrum exhibiting spina bifida occulta, the maxilla with supernumerary incisors, and cranial elements identified by Kieffer as showing porotic hyperostosis. The last sample was the fused vertebrae from the other individual thought to suffer from KFS from Lot VIII-13-367.

All pre-amplification analyses were carried out in laboratory facilities strictly dedicated to paleogenetic research following established procedures to prevent contamination (Cooper and Poinar, 2000; Kemp and Smith, 2005; Fehren-Schmitz et al., 2014). Samples cut from each of the 13 bones were treated with bleach to remove contamination from previous handling and ground to a powder for DNA extraction. Two separate DNA extracts were generated for each sample following an extraction protocol suitable for the recovery of short, degraded DNA fragments (Dabney et al.

2013). To determine mitochondrial haplotypes for the samples we amplified a 388 base pair fragment of the mitochondrial hypervariable region 1 (HVR1) [nucleotide positions 16021–16408, relative to the revised Cambridge Reference Sequence (rCRS)] using four pairs of overlapping primers. Two polymerase chain reactions (PCR) for each of the mitochondrial genetic marker were performed from each extract in order to corroborate results. Negative controls were employed for all PCR experiments as well as extraction blanks for the extractions. Amplification success was evaluated using 2.5% agarose gels and downstream sequence analyses followed by direct Sanger sequencing on an Applied Biosystems 310 Genetic Analyzer. Details for PCR protocols and analyses employed can be found in Fehren-Schmitz et al. (2010) and Fehren-Schmitz et al. (2011).

The only samples that produced DNA were the two fused vertebrae (VI-02B-118 and VIII-13-367) and a frontal bone fragment (VI-02B-33). Nevertheless, our paleogenetic analysis confirms earlier osteological analysis that at least two distinct adult individuals are present in VI-02B. While both VI-02B-33 and VIII-13-367 are assigned to haplogroup A2, the presence of a C at nucleotide position 16209 for sample VI-02B-118 indicates that it belongs to a different maternal lineage (A2q) [see Table 1]. We confirmed the consensus genotypes for the individuals using several independent replications (multiple PCRs from two independent extracts) to prevent false positive SNP determinations due to DNA damage or contamination. The genetic distance between these two distinct haplotypes, therefore, suggests that these individuals are not matrilineally related. This example indicates that the assignment of all bones with pathological traits to a single individual was incorrect. All employed negative controls and extraction blanks showed no amplification of PCR products.

Kieffer (2015a) had also speculated that the two individuals with KFS were closely related. Our determination of haplotypes, however, indicates that these individuals were not matrilineally related. While this does not preclude the possibility of a relationship between the individuals, it does allow us to move from the realm of speculation into that of established fact.

We were also able to determine that the vertebrae (VI-02B-118) belonged to a male by successfully amplifying Y-Chromosomal DNA. While not employed for the current question, an accurate estimation of sex could be valuable for determining MNI from commingled mortuary contexts. The ability of paleogenetics to establish biological sex from non-diagnostic skeletal elements may also prove valuable to osteoarchaeological studies in the future.

Finally, the re-examination of the cranial fragments from Lot VI-02 by Alison Galloway (Personal communication, 2015) found normal patterns of bone density and thickness consistent with aging individuals rather than porotic hyperostosis. Thus, the presence of porotic hyperostosis as an argument for type I KFS can be eliminated.

3.4 Conclusions

Skeletal and DNA preservation in sub-tropical environments like the southern Maya lowlands is notoriously poor. While poor preservation prevented us from establishing possible relationships between all the bones in question, we are encouraged by the results given the substantial degradation specific to working with ancient Maya skeletal material.

Kieffer (2015a) had raised the possibility that a number of bones exhibiting pathology might all belong to the same individual. This hypothesis, however, is not supported by our findings that a minimum of two individuals exist among the bones tested. Instead, our results reinforce Prout's (2015a) contention that there is no evidence for a condition more serious than type II KFS and that the individuals were most likely asymptomatic and, therefore, not sacrificed as "social outcasts."

From a methodological perspective, the minimization of the complexity and diversity within the lot created a plausible context for suggesting that all of the pathological elements belonged to a single individual. The genetic analyses, however, were unaffected by the high level of complexity and ambiguity in the context. There are a number of reasons that destructive analyses on human remains may be inappropriate, unsuitable, or prohibitively expensive. However, when paleogenetic approaches can be employed, they have the ability to help clarify more ambiguous data that can result from the osteological analysis of poorly preserved, commingle or immature human remains. This paper clearly demonstrates that applying paleogenetic methods to uncertainties of MNI and other challenging archaeological contexts can make osteoarchaeological publications more rigorous.

The recovery of DNA from the two fused vertebrae is promising. Further work employing target enrichment of genome-wide makers or those associated with the various forms of KFS combined with high throughput sequencing will allow us to refine the interpretations made here. While DNA preservation may remain an inhibiting factor for genome-level work at MTC, the speed of technological progress in the field of aDNA may yet provide us with methods for genetic paleopathology in the near future.

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CHAPTER 4

An Investigation of ancient Maya Intentional Dental Modification Practices at Midnight Terror Cave using Anthroposcopic and Paleogenomic Methods

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Authors: Cristina Verdugo*[a], Kimberly Zhu[b], Kalina Kassadjikova[a], Lara Berg[c], Jannine Forst[a], Alison Galloway[a], James E. Brady[d], and Lars Fehren-Schmitz[a]

[a] University of California, Santa Cruz, UC Paleogenomics, Department of Anthropology, 1156 High Street, Santa Cruz, CA 95064, USA

[b] University of California, Los Angeles, Department of Anthropology, 375 Portola Plaza, Los Angeles, CA 90095, USA

[c] Eberhard Karls Universität Tübingen, Interfaculty Institute for Cell Biology, Geschwister-Scholl-Platz, 72074 Tübingen, Germany

[d] California State University, Los Angeles, Department of Anthropology, 5151 State University Drive, Los Angeles, CA 900032, USA

Key Words: Dental modification, Ancient Maya, Paleogenomics, Midnight Terror Cave

Abstract: Evidence of intentional dental modification practices has been found throughout Mesoamerica dating from the Early Preclassic Period to the conquest. The recovery of 102 modified teeth from Midnight Terror Cave (MTC) provides a sufficiently large sample to critically examine current explanations of intentional

dental modification. Paleogenomic analysis was employed in order to test hypotheses which link intentional dental modification to sex and kinship. DNA was extracted and genomic sequencing libraries were made for 27 teeth. Results show the presence of both sexes, indicating that the practice is not sex linked. The mitochondrial genome data detects a possible link between intentional dental modification and style.

4.1 Introduction

Intentional dental modification is defined as changes to the natural morphology of a tooth for social or aesthetic reasons as opposed to changes due to wear or damage from repeated movements between teeth or between teeth and other materials. The practice has been documented in numerous cultures throughout the world (Labajo González et al., 2007; Tiesler, 1999; 2001; 2003; Fabian and Mumghamba 2007; Finucane et al. 2008; Wasterlain et al. 2016). Cross culturally, explanations for the practice include: initiation rituals and rites of passage; vanity or aesthetics; intimidation of enemies; totemic imitation; a means of mourning a loved one; cannibalistic reasons; tribal identity; avoidance of evil influences; avoidance of dental disease; prevention of lockjaw; and more practical reasons relating to the use of teeth as tools (Mower, 1999).

In Mesoamerica, evidence for the practice dates to the Early Preclassic Period continuing into the 16th century when it was noted by Diego de Landa (Romero, 1970; Tiesler Blos, 2001). Scholars posit a number of possible explanations for the practice focusing on aesthetics, rites of passage, and religious beliefs. More recently, Scherer (2018) posits that intentional dental modification served as a metaphor for social development and marked the transition to adulthood. Because the portion of the population with intentional dental modifications in Maya society is relatively small, most explanations focus on function, arguing that those with intentional dental modification are marked as distinct from those without. The most persistent hypotheses focus on its use as a social status indicator, for defining local family affiliation, or indicating lineage ties (Becker, 1973; Labajo González et al., 2007; Lopez Oliveres, 1998; Romero, 1958; Tiesler, 2001; Williams and White, 2006).

This study analyzes 102 modified teeth recovered from Midnight Terror Cave (MTC), Belize. Ancient DNA was analyzed from 41 teeth to determine the genetic sex and to test hypotheses which link intentional dental modification to sex and kinship. We prepared genomic sequencing libraries and in solution hybridization capture of the mitochondrial genome for 23 individuals. We were able to sequence full mitochondrial genomes for 17 individuals, the largest collection from a single site in Mesoamerica thus far. The size provides opportunities to critically examine hypothesis that link intentional dental modification to sex and kinship.

4.1.2 Background

Intentional dental modification is found primarily in the maxillary and mandibular incisors and occasionally the canine (Evans, 1973; Fastlicht, 1948; Romero, 1970; Williams and White, 2006, Tiesler, 2001; Scherer 2018). These locations make the modification visible to the community. Though there are examples of intentionally modified deciduous teeth elsewhere (Wasterlain et al. 2016), intentional dental modification has only been found in permanent teeth among the ancient Maya (Scherer 2018). Scholars (Romero, 1970; Tiesler, 1999; 2001) suggest the practice is generally found in individuals over the age of fifteen.

Modification is created using either the filing or inlay techniques (Havill et al., 1997). Alteration to the surface or shape of the crown such as notches, grooves, or points are made using the filing technique (Williams and White, 2006; 139). Dental inlays, appearing during the Middle Preclassic period, involves “the drilling of holes and [insertion] of various materials therein” (Williams and White, 2006: 139). Despite extensive evidence for these practices, there is little historical documentation detailing techniques for achieving an inlay dental feature (Williams and White, 2006).

Modification styles are often classified according the Romero system (1958; 1960; 1965; 1970; 1986), though earlier iterations by other scholars exist (Rubín de la Borbolla, 1940; Delfino, 1948). Romero (1970: 50; 52) identified three modes of modification which include: (a) alteration of the contour of the dental crown, (b) alteration of the labial surface, and (c) alteration of both the contour of the crown and the labial surface. Each of these modes is further subdivided into seven styles. Style A shows modification to the occlusal edge only while Style B shows modification to one of the angles of the crown. Both angles of the crown symmetrically modified are included in Style C. Modification showing straight filed lines on the labial surface of the crown, or scoring is considered Style D. Intentional dental modification for Style E, produces an inlay such as jade, pyrite, turquoise, or gold. Asymmetrical modification of both the occlusal edge and angles of the crown or, both the occlusal edge and labial face of the crown are characteristics featured in Style F. Finally, Style

G is composed of teeth showing both inlay and either symmetrical or asymmetrical alteration of the occlusal edge or angle.

Since Romero’s work, scholars (Buikstra and Ubelaker, 1994; Lopez Olivares, 1998; Tiesler, 2001; Whittington, 1998) have expanded the initial typology with the addition of previously unrecorded examples recovered from field excavations. Though Romero’s (1970) work focused primarily on Mexico, his classification system and technique have been applied throughout Mesoamerica.

4.1.3 Midnight Terror Cave

Midnight Terror Cave, located eight miles south of Belmopan in the Cayo District of Belize, was investigated from 2008-2010 as part of the Western Belize Regional Cave Project under the direction of Dr. Jaime Awe (Figure 1). The cave is located within a half kilometer of the major site of Tipan Chen Uitz. Architecture is found both on top of and at the base of the hill in which the cave is located, suggesting that the cave was incorporated into the surface site. The investigation of MTC, directed by James Brady, documented extensive architectural modification in the form of a platform at the entrance to the cave, two plazas within the cave, and two leveled areas surrounded by terraces in the lowest chamber. These constructions were designed to create level spaces that could be utilized for large public rituals as suggested by Inomata (2006). Several radiocarbon dates indicate that the modifications were initiated in the Early Classic (250 – 550 C.E.). While a substantial Early Classic ceramic assemblage was recovered, the majority of the 30,000 sherds date to the Late Classic period [550-900 C.E.] and a small number date as early as the Middle Preclassic [1000-400 B.C.E.].

Figure 9. Location of Midnight Terror Cave, Belize.

MTC is best known for producing a skeletal assemblage of over 10,000 elements. Initial studies suggest that the MTC assemblage was derived from sacrifice, given signs of trauma on bone, the archaeological context, and subadult demographics (Brady and Kieffer, 2012; Prout and Brady, 2017). Two radiocarbon dates of bone collagen place the individuals in the Late to Terminal Classic. The human bone is concentrated in two sections of the cave (Operations V and VIII), which correspondingly produced only limited amounts of ceramics. Thus, it appears that the cave was laid out around three large public spaces connected by constructed pathways to form a circuit. The substantially different artifact inventories suggest that these public spaces played host to different types of rituals.

4.2 Material and Methods

4.2.1 Materials

The MTC dental assemblage contains 1182 teeth, in total, composed of incisors, canine, premolars, and molars. Of the 337 incisors in the collection, this study focused on the 102 modified incisors. The majority of incisors are in relatively good condition, although some are covered with calcium carbonate from cave drip water, show brown or black discoloration from clay and manganese staining, and have missing or damaged roots.

The skeletal remains from MTC are highly fragmented and co-mingled. As a result, teeth were displaced from maxillae and mandibles and unassociated with specific individuals. The modified teeth come from Operations V and VIII within the cave (See figure 2). It is in these areas of the cave where the large majority of skeletal material was deposited.

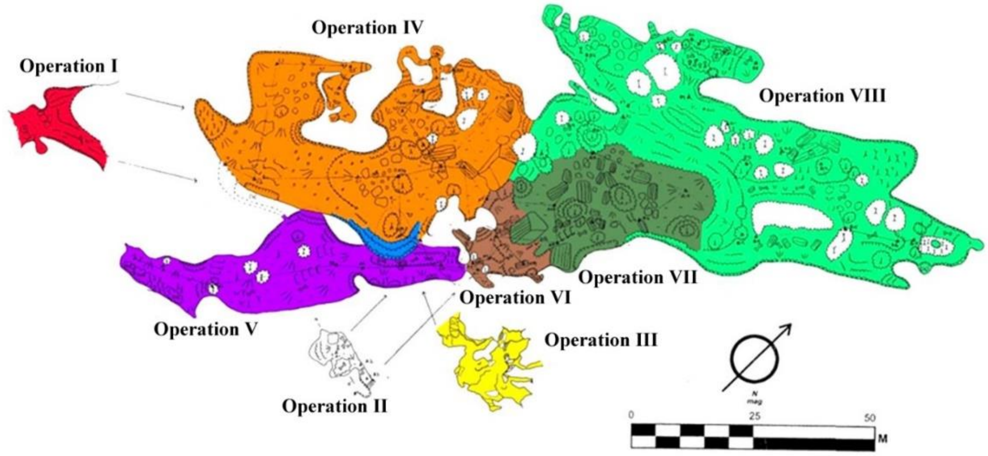


Figure 10: Map of Midnight Terror Cave

4.2.2 Methods

Teeth were cleaned using a dry toothbrush to remove excess soil. Each tooth was then identified within the dental arcade and, when possible, sided. This includes differentiating between the maxilla and mandible, as well as between central and lateral incisors. Additionally, teeth were examined both macro and microscopically to differentiate between breakage and intentional dental modification identified via tool marks.

For this study, the mesiodistal diameter of the crown and buccolingual crown diameter of each incisor was measured using a digital caliper (Hillson, 1996; White et al., 2011). These morphometric measurements were obtained to preserve dimensions of the teeth lost due to destructive analysis (See Supplementary Table 1).

The style, location of the style, and technique used to create the intentional modification on each tooth were compared to the Romero classification system

(1958; 1960; 1965; 1970). This involved examination and determination by one observer, then corroborated by a second researcher (See Supplementary Table 1).

Prior to destructive analysis, 31 teeth were photographed using a Nikon D3100 in a light box using a 360-degree photography turntable. A photograph was taken of the tooth every 10 degrees (see Supplementary 2 for examples). These images were then edited using Adobe Lightroom to unify color across all images for accuracy. Finally, the images were used to create 3D photogrammetric models using Agisoft Photoscan Professional software analyses in order to ensure that a permanent model is still available (see Supplemental data). For 10 samples, analyzed in 2015, photographs were obtained prior to destructive analysis.

As previously noted, biological sex estimation was impossible based on tooth morphology and due to lacking maxilla/mandibles which have a higher accuracy for sex estimation than teeth alone. However, estimating sex is important because of its relevance in intentional dental modification practice hypotheses. We successfully extracted DNA from 27 intentionally modified incisors in order to determine the biological sex of the individuals from which the samples derive, as well as to test for matrilineal relatedness.

Sample testing began with right maxillary I1 because they are the most abundant intentionally modified teeth in the MTC assemblage. This was followed by left maxillary I2. In order to obtain the required number of samples for hypothesis testing, sample testing expanded to include mandibular right I1 and left I2. This approach was combined with the cave geography in order to avoid potentially sampling the same individual twice. During the cave survey, operations were further subdivided into lots to maintain spatial control during mapping and surface collection. When possible, samples used in DNA testing were taken from different lots. While this could not guarantee the same individual would not be sampled more than once, it did provide some control in sampling the material.

To examine kinship, we employed a two-part strategy for DNA testing. We first tested samples which share modification style. Seven of the samples tested had the B5 modification style while five are C3. This would provide insight into the link between modification style and kinship. We, then, sampled teeth with different modification styles in order to identify genomic similarities/patterns present between styles.

Paleogenomic testing was conducted at dedicated clean room facilities of the Human Paleogenomics Laboratory at the University of California, Santa Cruz following established procedures to prevent contamination (Fehren-Schmitz et al., 2014; Llamas et al., 2016). Laboratory tools used to process samples were either sterile or decontaminated with full strength household bleach (6%) and exposed to UV light for 1 hour before use.

Tooth roots were cut using a diamond cutting disc, treated with bleach to remove contamination from previous handling, and pulverized using a mixer-mill as described in Fehren-Schmitz et al. (2014) for DNA extraction. Two separate DNA extracts were generated for each sample following an extraction protocol suitable for the recovery of short, degraded DNA fragments (Dabney et al., 2013). As suggested by Boessenkool et al. (2016), we employed an additional 15-minute pre-digestion step during the lyses protocol in which bone powder was mixed with 0.5% bleach solution. For each extraction, we used between 100-120 mg of pulverized tooth powder. Each extraction batch was accompanied by at least one extraction blank.

Partially UDG treated double barcoded double stranded DNA libraries were constructed for all samples following the protocol by Rohland et al. (2015). The success of the library construction, quantity, and length was evaluated using a TapeStation 2200. Each library was sequenced on an Illumina MiSeq sequencer at 1% (~300,000 reads) using the 2x75 paired end mode to evaluate library quality. Base-calling was performed using the Illumina software CASAVA 1.8.2. Raw reads were assigned to the corresponding samples based on the index sequence included in the adaptor P7, allowing for no mismatches. Forward (R1) and reverse (R2) reads were then further sorted and filtered by identifying those with the correct internal barcodes and discarding those without allowing up to one mismatch in the 7bp barcodes. Using an in-house script (https://github.com/mjobin/batpipe), adapters were trimmed, and reads were merged and mapped to the human reference genome using the software Burrows-Wheeler Aligner (BWA) version 0.7.5a-r405 7, with default parameters and seed option disabled (-l 1000). All raw sequence data processing steps and parameters were followed as described in Fehren-Schmitz et al. (2017) We used hg19 (GRCh37 build) as a reference genome, excluding the mitochondrial contigs. Mitochondrial reads were mapped to the revised Cambridge Reference Sequence (rCRS, NC_012920; 8) employing the same BWA parameters.

The pre-indexed and barcoded sequencing libraries that showed sufficient DNA preservation were then enriched for mitochondrial DNA using the MYbaits Mito Human-Global bait set for in-solution hybridization capture (MYcroarray, Ann Arbor, MI). Libraries were captured following the manufacturer's instructions (http://www.mycroarray.com/pdf/MYbaits-manual-v3.pdf). The captured libraries were amplified for 20 cycles with IS4 and indexed P7 primers as described above. Subsequently, libraries were purified with AMPure XP beads and quantified by 2200 TapeStation (Agilent Technologies). Sequencing was carried out on an Illumina MiSeq sequencer in paired end (2x75 bp) mode, at ~600,000 reads per sample.

To evaluate the authenticity of our read data, we assessed the damage patterns to see if they were characteristic of ancient DNA. Since our libraries were partially UDG-treated, most of the damage accumulating at the ends of the molecules except the terminal bases was removed by the enzyme. However, terminal CpG dinucleotides are unaffected by the UDG treatment when methylated. We estimated patterns of DNA damage using PMDtools 10 and observed that damage for all samples exceeded 11% as to be expected for ancient DNA samples (Rohland et al., 2014). We further estimated mitochondrial contamination rates by employing the modules contDeam and mtCont implemented in the software tool SCHMUZTI using the recommended parameters (Renaud et al., 2015).

Sex was determined by evaluating the ratio (Ry) of reads aligning to the Y chromosome (nY) compared to the total number of reads aligning to the sex chromosomes (nX + nY), i.e., Ry = (nY/nY+nX), as described in Skoglund et al.

(2013). In addition, we employed the X-chromosomal normalization rate (Rx) approach introduced by Mittnik et al (2016) that compares Rxto the variability observed in all 22 autosomes, which promises higher accuracy for sex determination, especially when dealing with only few reads. Both sex determination methods were applied to the read data from both the initial shotgun screening data and the reads from the mitochondrial capture.

To identify the mitochondrial haplotypes of the sequenced individuals, we conducted a manual analysis as described in Llamas et al. (2016) rather than relying on automated procedures discussed in other papers. For each of the eight sequenced libraries all mitochondrial reads mapped to the rCRS using BWA were visualized in Geneious v7.1.3 (Biomatters; available from http://www.geneious.com/) for each sample. The assembly and the resulting list of SNPs were verified by eye and compared to SNPs reported at phylotree.org (mtDNA tree Build 17 [18 Feb 2016]) (van Oven, 2015). Following recommendations in van Oven and Kayser (2009), we excluded common indels and mutation hotspots at nucleotide positions 309.1C(C), 315.1C, AC indels at 515–522, 16182C, 16183C, 16193.1C(C), and C16519T. Haplotype motives for 17 samples for which we were able to generate full mitochondrial consensus genomes can be found in the Supplementary Table 3. All new mitochondrial data presented in this study are available at the National Center for Biotechnology Information (NCBI) under the accession.

numbers MN848572 to MN848588.

4.3 Results

The MTC material, for the most part, is well accommodated within the Romero classification. Fifteen different modification styles were identified in the assemblage. Figure 3 illustrates the various styles and frequencies in which they appear. The most common styles include A2 (19 examples), B5 (19 examples), and C3 (19 examples), collectively comprising about 56% of the total sample. Style C9, with 12 samples, is also a popular style. Styles A4, B1, C5, F2, and F10 are each represented by a single example. The remaining samples include 3-7 examples per style (see Figure 3). Two of the teeth could not be classified according to Romero's typology. These teeth show signs of filing, which may have occurred after initial intentional modification, as well as additional modification unattributable to tools or methods used for intentional dental modification.

Forty-one modified teeth were tested for DNA preservation. Twenty-seven samples were successful for DNA extraction and amplification and were made into sequencing libraries. The shotgun screening sequencing statistics outlining the endogenous DNA content, complexity of molecules retained, damage patterns, contamination rates, and genetic sex can be found in the Supplementary Table 4. Based on the preservation screening results we selected 23 sequencing libraries for the mitochondrial in-solution enrichment (see Supplementary Table 4). We were able to sequence complete mitochondrial genomes and determine the haplotype for 17 of the sequencing libraries.

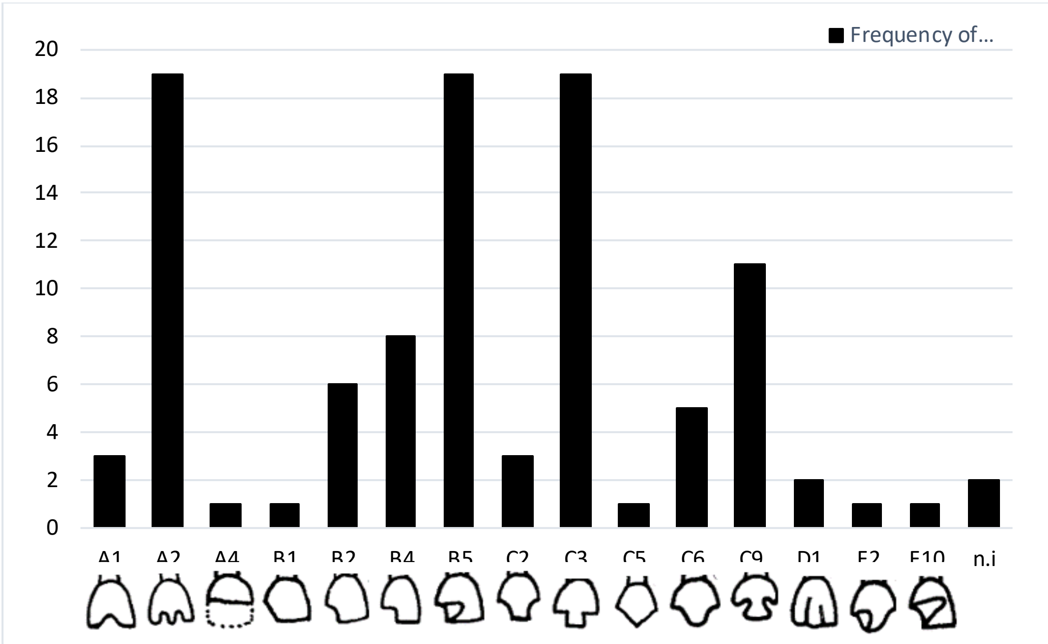


Figure 11. Frequency of Intentional Dental Modification Style

Results from the shotgun sequencing and mitochondrial genome capture demonstrate the presence of both males and females (Table 1). Shotgun sequencing results identified 16 females and 7 males. Sequencing reads obtained from the mitochondrial genome capture produced sufficient data for sex determination for 19 sequencing libraries; 12 samples were determined to be female and 7 samples were determined to be male. The remaining samples either were not analyzed in the mitochondrial genome capture or not enough data was obtained to determine sex. Where sex was identified in a sample using both techniques, there was an 89% match with two samples (VIII-15A-2001; VIII-16A-2009) producing mismatching results. Three samples (VIII-16A-2004; U.P.-1-2002; VIII-7C-2002) produced enough data to determine the sex, but not the mitochondrial haplotype.

Table 1. Haplotype and Sex Determination Paleogenetic Results

Sample	Modification Style	mt Haplotype	Shotgun Seq Sex ID	MtCapture Sex ID
V-01E-2003	B2	not analyzed	XX	not analyzed
V-01E-2004	A2	not enough data	XX	not enough data
V-01E-2005	C9	not analyzed	XY	not analyzed
V-01G-2001	C2	A2	XY	XY
V-03G-2001	C2	A2r	XY	XY
VI-01A-2001	C3	not analyzed	XX	not analyzed
VI-01A-2002	C3	A2	XX	XX
VIII-7C-2002	C9	not enough data	XX	XX
VIII-8C-2004	B5	A2	XX	XX
VIII-11C-2001	A2	A2h	XX	XX
VIII-13-2001	B5	B2	XX	XX
VIII-13-2006	B2	not enough data	XX	not enough data
VIII-13-2007	B5	not analyzed	XX	not analyzed

VIII-13-2008	B5	A2m	XY	XY
VIII-13-2009	C3	A2q	XX	XX
VIII-13-2012	B5	A2m	XY	XY
VIII-13-2015	C3	B2	XX	XX
VIII-14B-2002	A2	not enough data	not enough data	not enough data
VIII-14B-2004	B5	C1c4	not enough data	XX
VIII-15A-2001	C6	A2	XY	XX
VIII-16A-2001	B2	A2r1	XX	XX
VIII-16A-2003	B4	A2	XY	XY
VIII-16A-2004	A2	not enough data	XX	XX
VIII-16A-2005	B4	A2	XY	not enough data
VIII-16A-2009	C3	A2	XX	XY

U.P-2002	B5	not enough data	XX	XX
U.P-2003	C3	A2	not enough data	XY

*Two samples are designated as "U.P." because they are missing original provenance.

With the mitochondrial genome capture we obtained sufficient coverage for 17 individuals to reconstruct complete mitochondrial genomes and determine the haplotype (see Supplementary Table 3 for haplotype motives). Fourteen samples are identified in the A2 haplogroup, 2 in haplogroup B2, and 1 in haplogroup C. Detailed information of each identified haplotype can be found in Supplementary Table 3. A majority (11/14) of the individuals belonging to haplogroup A2 exhibit private variants not shared with other A2 individuals in the studied population, which means that none of the 11 individuals share the same A2 haplotype (see Supp. Tab. 3). This demonstrates that these are unique individuals, not directly maternally related to one another. Two samples (VIII-13-2008 and VIII-13-2012) belong to the same A2m haplogroup. The presence of three variants for sample VIII-13-2012, however, indicates different haplotypes showing that both teeth are from separate individuals and do not share maternal ancestry.

We have five samples which share the same haplotypes and, therefore, are maternally linked (see Supplementary 5). Three samples (VIII-16A-2009, VIII-16A-2005, VIII-16A-2003) share a haplotype A2+(64). Sequencing results also show that two samples (VIII-13-2001; VIII-13-2015) share a B2 haplotype. This point will be further discussed below.

4.4 Discussion

It has been suggested that intentional dental modification serves as identifying markers of status within a community. If that is the case, then intentional dental modification practices could give an important insight into who was sacrificed at MTC and how they were selected. The most obvious criteria that we can address with current information have to do with differences by sex or ancestry. The 17 mitochondrial genomes fall into the three haplogroups: A, B, and C. Most, 82%, belong to haplogroup A2, 12% to haplogroup B2, and 6% to haplogroup C1. These haplotypes (A2; B2; C1) are commonly identified in both ancient and modern Mesoamerican populations (González-Martin et al., 2015; Mizuno et al., 2014; Ochoa-Lugo et al., 2016; Sandoval et al., 2009).

Based on the results, we estimate the minimum number of individuals (MNI) analyzed in this study to be 17. This MNI is derived through the tooth, modification style, physical location within the cave, and paleogenomic data. As previously noted, we believe that teeth sampled from different locations within the cave belong to different individuals. This point, however, would be confirmed via paleogenomic analysis. Additionally, we considered the tooth, taking into account its numbering and side within the dental arcade. The modification style was also used to discern between individuals. Many examples illustrate that the maxillary central incisors often mirror one another in modification style (Romero 1970: 54-55, Figure 3-4; Tiesler 2000: 47;

Dufoo Olivera et al. 2010: 100 Figures 1-3) and, therefore, was used as criteria for selecting samples from different people.

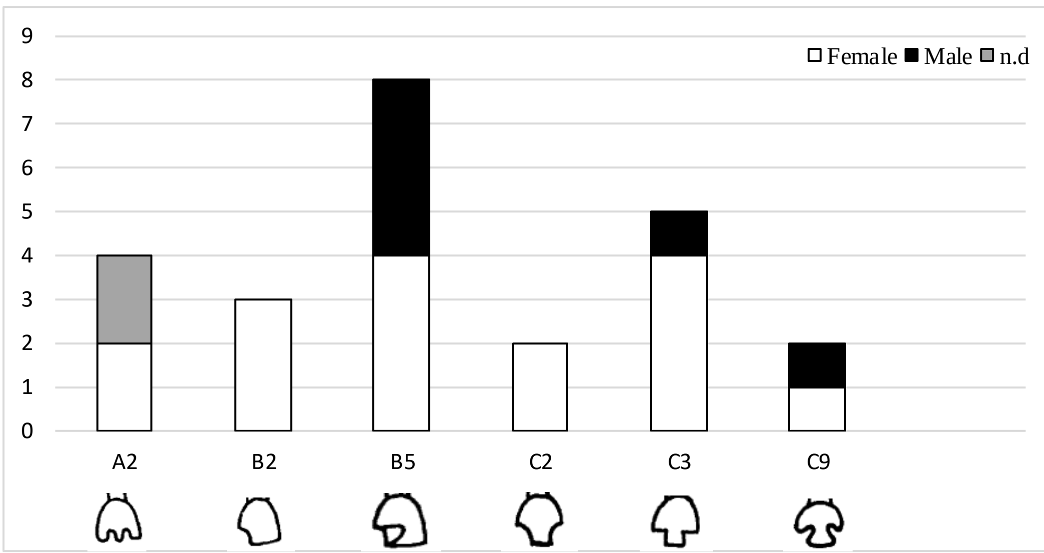
Our analysis demonstrates that there is no overlap between individuals in different operations. When examining paleogenomic data from each lot (smaller subdivision of the Operation), we identified the aforementioned two samples (VIII-13-2001; VIII-13-2015) that could come from one person. Because they have different modification styles, we believe it is unlikely they came from the same individual.

4.4.1 Linking Sex and Intentional Dental Modification

There is little question that at least part of the explanation for intentional dental modification is aesthetic so certain styles may be regionally or temporally popular. López Olivares (1997; 1998) identified styles E, F, and G as appearing more frequently in Guatemala and the Petén region. It is concluded that individuals living at different sites may have particular style preferences.

Aesthetics can vary culturally by gender; therefore, it seems appropriate to ask if the practice or specific forms are sex-linked. Clarification must be made that “sex” and “gender” are related but not equivalent. The results of our paleogenomic analysis demonstrate the presence of both sexes within the MTC collection. Table 2 shows the correlation between modification style and sex and demonstrates that both sexes shared modification styles. Styles A2, B2, and C2 are only represented by females and so could be sex linked. We would caution, however, that the sample size is small, and the tendency is for styles to be shared by both males and females. We are reluctant, therefore, to say that modification styles are sex-linked, but we note these results so that they can be checked by future research. Numerous scholars (Havill et al. 1997; Massey and Steele, 1997; Williams and White, 2006; Saul and Saul, 1997; Scherer, 2018) also reports finding the practice of intentional dental modification in both males and females in near equal numbers throughout the Maya area.

Table 2. Modification Style and Sex



The question of sex is additionally important because the MTC assemblage is argued to be the result of human sacrifice (Brady and Kieffer, 2012; Prout and Brady, 2018). While it is well recognized that intentional dental modification was practiced by both sexes, a sacrificial assemblage has not been previously examined. Many hypothesize that young males captured in battle were the most common sacrificial victim, in part because Maya iconography consistently shows males fighting, being captured, and being sacrificed (Coe and Kerr 1997:35, 58). It is important to establish that not only are both sexes present at MTC but that more than 60% of our sample is female. Thus, iconography focusing on males may show an androcentric bias not sustained by actual data. Clearly the young male warrior model of human sacrifice needs to be rethought.

4.4.2 Group Affiliation

It has been suggested that modification style may have identified individuals as members of a social group such as a lineage or clan (White, 1994; Tiesler Blos, 2001). This theory suggests that members or perhaps higher status members of particular lineages would bear modifications that marked their membership in a kin group. The high frequency of styles A2, B5, C3, and C9 at MTC is consistent with this concept. In their work at Lamanai, Belize, Williams and White (2006), show that a number of Maya sites (Cuello, Uaxaxtun, Barton Ramie, Southeastern Peten, Lubaantun, Piedras Negras, Colha, Tipu, Chau Hiix) share at least one of the same high frequency styles identified at MTC. This could suggest kin groups or elite families crosscutting individual polities.

It should be noted that when the idea that intentional dental modification was proposed as marking family or kin group affiliation there was no way of testing such propositions. Within MTC, mitochondrial genome analysis allows us to take the first steps in comparing modification style with haplotype, potentially addressing the link between style and kin group affiliation.

An interesting case was encountered when examining paleogenomic data from each lot. As previously noted, we identified three samples sharing the same haplotype, A2+(64), (VIII-16A-2003, VIII-16A-2005, VIII-16A-2009) and two samples (VIII-

13-2001; VIII-13-2015) sharing the same B2 haplotype that could come from the same individuals. Two of the haplotype A2+(64) samples (VIII-16A-2003; VIII-16A-2005) were determined as male and one was determined as female (VIII-16A-2009). Sample VIII-16A-2003 is a right central maxillary incisor (I1). Sample VIII-16A-2005 is a left central maxillary (I1). These two samples then, are both male, have the same B4 modification style, and share maternal ancestry. It is possible, therefore, that these two samples may come from the same individual. However, it is interesting that the third sample, a female, with the same haplotype (VIII-16A-2009) is also maternally related but shows a different modification style. Additional DNA testing is required to distinguish these samples from one another.

The samples sharing the same haplotype B2 are both determined to be female. Sample VIII-13-2001 is a central maxillary incisor (I1). Sample VIII-13-2015 is a lateral maxillary incisor (I2). At this point, we are unable to distinguish this these sample set without further DNA testing. However, the two samples also exhibit different modification styles (B5 and C3, respectively) so on these grounds we are inclined to believe that they are different individuals. If this is the case, we have two closely related individuals with different modification styles. If they are shown to come from the same individual, it complicates the analysis by having multiple styles in a single mouth. This would undermine a fundamental assumption in most of the literature on dental modification that styles are separating groups from one another.

It is important to note that the Maya are a patrilineal society which would make our mitochondrial focused testing insufficient to make a clear argument for linking modification style and kinship. In examining only mitochondrial genome data, we are unable to test whether modification style and kinship is linked through male decent. We are awaiting results from high-sequencing analysis currently being performed on the samples. With this data, we will have a more complete picture of the relationship between intentional dental modification style and consanguineous kinship.

4.5 Conclusions

While the function of intentional dental modification remains somewhat elusive, the Midnight Terror Cave dental assemblage provides an opportunity to examine a number of the common assumptions. The application of paleogenomic analyses provides data that has been unavailable in previous studies and which has been indispensable in dealing with the MTC dental assemblage. Because this is the first sacrificial assemblage analyzed, it was uncertain if our results would differ radically from those obtained from traditional mortuary assemblages. Our ability to determine sex genetically allowed to us verify that, as with other sample populations, both sexes practiced dental modification. At MTC, however, the fact that more than 60% of our sample is female has additional implications in that this high percentage conflicts with models that predict that most victims are males conscripted as war captives.

Our attempts to test models of group affiliation were frustrated by our use of mitochondrial DNA which is handed down matrilineally in a patrilineal society. Nevertheless, the data produced several cases that bear closer scrutiny. In this case, we have two sets of closely related individuals. One set share the same modification style, the other set has different modification styles. It is possible that the two samples come from the same individual but if individuals display multiple styles, the relationship between styles and social groups may become too complex to disentangle. As we apply genome-wide analyses to our samples, it will become possible to better address questions regarding the relationship of styles to kinship groups.

4.6 Acknowledgements

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CHAPTER 5

Implications of Age and Sex Determinations of Maya Sacrificial Victims at Midnight Terror Cave

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Authors: Cristina Verdugo*[a], Kimberly Zhu[b], Michael Prout[c], Nasreen Broomandkhoshbacht [a], Alison Galloway[a], Lars Fehren-Schmitz[a], and James E. Brady[c]

[a] University of California, Santa Cruz, Human Paleogenomics Laboratory, Department of Anthropology, 1156 High Street, Santa Cruz, CA 95064, USA

[b] University of California, Los Angeles, Department of Anthropology, 375 Portola Plaza, Los Angeles, CA 90095, USA

[c] California State University, Los Angeles, Department of Anthropology, 5151 State University Drive, Los Angeles, CA 900032, USA

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Abstract: The prurient element in the popular notion of the Maya sacrifice of “beautiful virgins” during the first half of the twentieth century (Frost and Arnold 1909; Willard 1926) appears to have made researchers wary of studying women in human sacrifice. Interest in human sacrifice arose in the 1990s along with the formulation of the warfare hypothesis for the collapse of Maya civilization so that models of human sacrifice often assumed that victims were largely male war captives.

The present study reports on the detailed examination of all the pelvises in the Midnight Terror Cave skeletal assemblage, using osteological and paleogenomic techniques to shed light on the age and sex composition of sacrificial victims. Our analysis demonstrates the presence of both males and females ranging from subadult to older adult ages. All four paleogenomic sex determinations on samples from subadults were determined female. Additionally, results indicate females fall into two age categories suggesting that sacrificed females may have served as deity impersonators in rituals dedicated to female deities.

5.1 Introduction

Discussions of ancient Maya human sacrifice produced varied models of the demographics of sacrificial assemblages. These models draw heavily from ethnohistoric literature due to the dearth of analyses of large sacrificial assemblages. Hypotheses regarding who was sacrificed differ in both age and sex with accounts detailing the sacrifice of children, men, and women in varying contexts. Midnight Terror Cave (MTC) produced a commingled osteological assemblage of over 10,000 skeletal elements, most belonging to sacrificed individuals (Brady and Kieffer, 2012; Prout and Brady, 2018). With the application of paleogenomics, MTC has the potential to advance our understanding of these assemblages.

This paper reports on the analysis of subadult and adult pelvic bone fragments (n=184) from the MTC skeletal assemblage to assess the demographic composition of this sample. Age of adult fragments was estimated using general age categories based on morphological age-related alterations to the pelvic bones occurring throughout life. Age for subadults was estimated through comparison of size and development to other groupings and via descriptions of immature bone material at different age ranges (Prout and Brady, 2018). When possible, sex was estimated using anthroposcopic techniques focused on sexual dimorphism. Sex, often, cannot be determined for subadults because the diagnostic traits appear only after puberty. However, paleogenomic analysis was conducted on 24 fragments, including four subadults, to confirm, augment, or correct interpretations obtained in the osteological analysis. This approach was useful for dealing with remains for which sex could not be estimated using anthroposcopic techniques due to poor preservation, calcium carbonate build-up around the bone, or where sexually diagnostic features are lacking.

5.2 Background

5.2.1 Maya Human Sacrifice

Bishop Diego de Landa's *Relación de las cosas de Yucatán* provided one of the earliest accounts of Maya human sacrifice. Landa describes the sacrifice of men and women in Chichen Itza's Cenote of Sacrifice for rain petitions (Tozzer, 1944). Edward Thompson's recovery of human remains from the cenote from 1904-1910 was seen as corroboration of Landa's accounts. Recovery of these remains provided the backdrop for 20th century thinking about the composition of sacrificed individuals in terms of sex and age.

The topic of sacrifice took on sexual overtones during the early 20th century, focusing on women. Arnold and Frost (1909: 92) speculated that young girls may have been defiled by high priests in a building above the Cenote of Sacrifice, surrendering their virginity and life to the Rain Deity. In *City of the Sacred Well*, T.A. Willard (1926:53) describes the sacrifice of a "flawless -most lovely maiden" and implies willing participation by the victim. As the topic garnered popularity, images depicting Maya women being thrown into the Cenote of Sacrifice were published in *National Geographic* (Herget 1936).

The perspective was so widely disseminated that Hooten (1940: 272-273) noted in his report on the Cenote of Sacrifice that though some perspectives idealized the sacrifice of virgins, osteological evidence could not permit the determination of the point. Although the popular view was never accepted within the anthropological community, the prurient overtones inhibited discussion of gender in sacrifice among serious scholars.

The discussion of the sacrifice of men draws heavily from Aztec models (Durán, 1972; Sahagún, 1951). Accounts of the dedication of the Templo Mayor describes the sacrifice of thousands of individuals, portraying the victims as captured warriors. With the rise of the warfare hypothesis for the Maya collapse in the 1990s (Demarest et al., 1997), the implicit assumption was that large-scale human sacrifice followed the Aztec model involving predominately male war captives. The warfare model was coupled with Maya iconography that depicted the capture of males in battle and being sacrificed (Coe and Kerr, 1997).

The sacrifice of children, although well-documented in ethnohistoric sources, has not been at the heart of theoretical discussions on ancient Maya sacrifice. Spanish sources illustrate the complexity of the recruitment issue. As Tozzer notes, “There seems to have been certain persons whose office (*oficio*) was to obtain children for sacrifice by kidnapping.” (Tozzer, 1944:117, n.535). Orphans were sold for sacrifice, children for sacrifice were given as gifts from one important person to another, and the devout might even donate their own children for sacrifice (Tozzer, 1944:117, n.535).

It’s important to note that there are numerous discussions of ancient Maya human sacrifice that outline evidence for the use of children and adults (Anda Alanís, 2007; Barret and Scherer, 2005; Beck and Sievert, 2005; Domenici 2014; Duncan, 2011; Geller, 2012; Houston and Scherer, 2010; Lucero and Gibbs, 2007; Massey and Steele, 1997; Prout and Brady, 2018; Saul and Saul, 1989; Scherer, 2017; Tiesler, 1998, 2007; Tiesler and Cucina, 2007). MTC provides a means of testing hypotheses about age and sex distribution in a large collection.

5.2.2 Midnight Terror Cave

MTC, located in the Cayo District of Belize, was the focus of archaeological investigation between 2008-2010 (Figure 1). Extensive architectural modification within the cave includes constructed plazas, terraces, and pathways which created level spaces that could be utilized for large public rituals. These spaces were accessible via a formalized circuit, directing visitors through the cave (Brady and Kieffer, 2012). Radiocarbon dating of charcoal from a series of superimposed floors places the construction during the Early Classic [250-550 C.E.]. Ceramics recovered from the surface of the construction likewise dates use to the Classic period with the heaviest utilization occurring during the Late Classic period [550-900 C.E.]. Radiocarbon dates obtained from two MTC bone fragments (V-01e-285 and VIII-13-367) confirm Late Classic period use; cal AD 676-772 and cal AD 775-886, respectively.

5.2.3 The MTC Skeletal Assemblage

The MTC skeletal assemblage matches a number of features identified by Berryman (2007) and Tiesler (2007) pertaining to post-sacrificial deposits. These features include remains associated with public or ceremonial space, lack of interment, signs of violence, lack of mortuary offerings, and clear selection for certain members of a population.

At MTC, the placement of bodies in highly visible or ceremonial spaces is exemplified throughout the cave. For example, Operation V, the deepest and wettest part of the cave, has a number of large terraces that could have accommodated several hundred people. Brady and Kieffer (2012: 251) argue that this space is associated with the public demonstration of sacrifice, mirroring Inomata's (2006) ideas of performance and power.

The skeletal material at MTC was found commingled and scattered across the cave floor, demonstrating a lack of interment. On a platform in Operation V, bone had been cleared and deposited in a seasonal pool so that only smaller bones, such as phalanges, were recovered on the platform. In one place along the ritual pathway, bones were left exposed and, in some cases, trampled. Operations V and Operation VIII with the heaviest concentrations of skeletal material both have differentiated space for public and private ceremonial activity (Figure 2).

While signs of violence do appear in the MTC skeletal assemblage, they are infrequent. The buildup of calcium carbonate on the bone from dripping water may obscure them as it has covered many subtle bone features and left many remains cemented to the cave floor. Still, Kieffer (2015) identifies 28 cases of perimortem trauma in the collection, including cutmarks.

Artifact concentrations vary throughout the cave. Spaces with a strong presence of human remains have minimal ceramics, indicating that these areas were used almost exclusively for sacrifice. Absent from MTC are artifacts resembling mortuary offerings (Prout and Brady, 2018; Brady and Kieffer, 2012).

Finally, the demographic profile of MTC suggests the selection of certain members of the group. Forty-three percent of the individuals are subadults and most died at ages when mortality is generally low (Prout and Brady, 2018). This pattern, like the mortality curve of the Cenote of Sacrifice, differs from a normal Mesoamerican mortality curve identified by Storey (1985) in her study of a residential unit in Teotihuacan. The MTC subadults align with ethnohistoric data identifying children of five to six years of age as most frequently selected for sacrifice (Prout and Brady, 2018). Additionally, these results are congruent with a larger pattern documented by Cucina and Tiesler (2014: 243) which identifies infant and subadult remains found "in caves, cenotes, and other natural underground places …

as clearly distinct from the natural mortality profile, denoting in some cases a cultural pattern involved in the preferential deposition of individuals in this age range".

5.3 Materials and Methods

This study arose from a general dissatisfaction with established procedures for determining sex ratios in a collection that is commingled, calcite covered, and, in which, intact skulls are rare. We estimated the sex ratio of the MTC assemblage through the examination of a single anatomical area (pelvis) which shows reliable morphological differences for sex and supplemented this with paleogenomic data. Remains range in size from complete bones to smaller fragments.

5.3.1 Materials

This study examined all pelvic material (n=184) in the MTC assemblage. Estimation of sex and age on the adult material was made only for ilium, ischium, and pubic bones or os coxa between 50-100% complete. Fragments smaller than this, while still catalogued and examined, were not estimated for sex or age. It is important to note that poor preservation of the material left many of the bones impossible to analyze due to postmortem breakage, discoloration, or coverage in calcium carbonate. Remaining fragments often consist of only one area useful for determining age or sex.

5.3.2 Sex Estimation

While numerous multivariate and morphometric approaches have been developed for sex estimation in the pelvis, anthroposcopic methods were applied in this study due to inherent problems with defining measurement landmarks exacerbated by the poor condition of the remains (Klales et al., 2012). Examined features include overall robusticity of the bone, subpubic angle, preauricular sulcus, ventral arc, subpubic concavity, and the greater sciatic notch. These features of the pelvic girdle have demonstrated sexual dimorphism identifiable through visual assessment (Buikstra and Ubelaker, 1994; Klales et al., 2012; Novak et al., 2012; Phenice, 1969; Walker, 2005).

5.3.3 Age Estimation

Age estimation is less precise in adults than subadults. Variation in the aging process occurs between individuals in the same population. For this reason, we utilized general age categories (early, young, middle aged, older, elderly) based on morphological traits associated with age rather than matching features to more narrowly defined age ranges derived from modern populations or attempting to seriate and assign ages (Brooks and Suchey, 1990; Lovejoy et al., 1985; Miranker, 2016; Rissech et al., 2006). Categories are defined in Table 1. For a description of the methodology employed in age-at-death estimation of subadults see Prout and Brady (2018).

5.3.4 Minimum Number of Individuals

The minimum number of individuals (MNI) is calculated based on duplication of elements within an assemblage. MNI is useful in commingled contexts, which lack discrete burials. Where possible, elements were sorted by side, age, sex, size, and both bilateral and non-metric traits to estimate the greatest MNI present (Byrd and Adams, 2009; Konigsberg and Adams, 2014; Osterholtz et al., 2014).

5.3.5 Paleogenomic Methods

Paleogenomic testing was conducted at the Human Paleogenomics Laboratory at the University of California, Santa Cruz in dedicated clean room facilities, following established procedures to prevent contamination (Fehren-Schmitz et al., 2014). The samples for paleogenomic testing were selected from different lots in MTC to ensure, as much as possible, that all samples belonged to different individuals. Samples were photographed before destructive analysis. Laboratory tools used were either sterile or decontaminated using full strength household bleach (6%) or Alconox (Alconox, Inc.) and exposed to UV light before use.

Approximately one gram of bone was cut from each sample, bleached to remove contamination, and ground to a powder following protocol for ancient DNA analyses (Fehren-Schmitz et al., 2014). Two extractions were produced from each sample. The first set of extractions were performed strictly following the Dabney et al. (2013) protocol. For the second set of extracts, we employed an additional 15-minute pre-digestion step during the lyses protocol in which bone powder is mixed with 0.5% bleach solution (Boessenkool et al., 2016). This approach has been shown to remove contaminating DNA on bone before lysis begins. Each extraction batch was accompanied by at least one extraction blank.

Partially UDG treated single stranded DNA libraries were constructed following the protocol by Troll and colleagues (2019) for all samples. Success of the library construction, quantity, and length was evaluated using the TapeStation 2200 (Agilent) and Qubit (Invitrogen). Each library was sequenced on an Illumina MiSeq sequencer for ~300,000 reads using the 2x75 paired end mode to evaluate library quality. Base-calling was performed using the Illumina software CASAVA 1.8.2. Raw reads were assigned to the corresponding samples based on the index sequence included in the P7 and P5 adapters, allowing no mismatches. Using an in-house script (https: //github.com/mjobin/batpipe), adapters were trimmed, reads merged, and mapped to the human reference genome using the software Burrows-Wheeler Aligner (BWA) version 0.7.5a-r405 7, with default parameters and seed option disabled (-l 1000). All raw sequence data processing steps and parameters were followed as described in Fehren-Schmitz et al. (2017). We used hg19 (GRCh37 build) as reference genome, excluding the mitochondrial contigs. Mitochondrial reads were mapped to the revised Cambridge Reference Sequence (rCRS, NC_012920; 8) employing the same BWA parameters.

We estimated patterns of DNA damage using PMDtools (Skoglund et al., 2014) and observed that damage for all samples was as expected for ancient DNA samples, ranging between 4-11% at the terminal bases (Rohland et al., 2014). We further estimated mitochondrial contamination rates employing the modules contDeam and mtCont implemented in the software tool SCHMUZTI using the recommended parameters (Renaud et al., 2015).

Following Skoglund and colleagues (2013), sex was determined by evaluating the ratio (Ry) of reads aligning to the Y chromosome (nY) compared to the total number of reads aligning to the sex chromosomes (nX + nY), i.e., Ry = (nY/nY+nX). We also employed the X-chromosomal normalization rate (Rx) approach introduced by Mittnik et al. (2016). This compares Rx to the variability observed in all 22 autosomes, promising higher accuracy for sex determination.

5.4 Results

5.4.1 Paleogenomic Results

Successful extraction and amplification of DNA from the pelvis is notoriously difficult because the bone is extremely porous. More compact bone is preferred for ancient DNA analysis as it is more likely to contain endogenous DNA. Of the 24 samples, DNA analysis was successful for 16. The sequencing statistics for the screening shotgun sequencing outlining endogenous DNA content, complexity of molecules retained, as well as damage patterns, contamination rates, and genetic sex can be found in the Supplement Table 1.

Of the 16 successful libraries, sex was determined for nine samples including eight females and one male. Three samples (SD-03-18, V-01e-346, and V-01e-343) exhibit morphologically female characteristics and are confirmed as female. Sample SD-03-75 was estimated as “male” via a narrower greater sciatic notch but determined to be female through paleogenomic testing. Sample V-01e-238, estimated to be early adult of unknown sex, was determined to be male. Shotgun sequencing for SD-02B-28 gave conflicting results for sex, likely due to the low amount of data.

The remaining four samples (V-01c-57; VIII-02a-398; VIII-04-92; V-01e-336) come from subadults and, therefore, sex could not be estimated using anthroposcopic analysis. Paleogenomic testing determined this material to be female. With these examples, paleogenomic data was able to determine sex of an inestimable skeletal element; adding to the overall demographic data used to answer hypotheses at the heart of this study.

5.4.2 Anthroposcopic Results

Our analysis identified 85 adult and 99 subadult pelvic bone fragments. Sixty-three left (17 adult; 46 subadult) and 57 right (16 adult; 41 subadult) elements were distinguished. Of the 85 adult bones examined, sex could not be estimated using morphology for 52 due to small fragment size, poor preservation, and post-mortem damage. Of the remainder, 10 fragments were identified as male and 22 fragments were identified as female. As previously noted, we were unable to sex subadult samples based on morphology.

Age was estimated for 120 fragments. All age ranges are present in the MTC assemblage except for "Elderly". This includes 87 subadults, six early adults, 11 young adults, 14 middle age adults, and two older age adults. Small fragment size, poor preservation, and post-mortem damage prevented age estimation of 64 fragments. Males fall primarily into the Young (1) and Middle Adult (4) age ranges.

Females appear in the Subadult (3), Early Adult, (4) Young Adult (5), and Middle Adult (8) age ranges. The two elements estimated to be “Older Adult” individuals could not be estimated for sex. Age distribution results of the subadult material can be found in Figure 3. Figure 4 illustrates the adult range ages. The results of the age and sex can are demonstrated in Figure 5.

When taking side, age, and sex, into account an MNI of 67 was estimated including 12 adults and 55 subadults. Our analysis identified five female and seven male adults. We urge caution in the accuracy of this estimation given the highly fragmented remains and reliance on a single skeletal element (Byrd and Adams, 2009).

5.5 Discussion

Our osteological and paleogenomic results provide new perspectives on the practice of sacrifice. That the four MTC subadult samples producing usable paleogenomic profiles are all female requires closer consideration. Ours is not the first study to examine sex of sacrificed subadults. De la Cruz and colleagues (2008) employ a PCR-based method for determining sex of 37 subadults and six adults recovered from Temple R at Tlatelolco, Mexico City. Molecular sexing identified eight males and one female. According to De la Cruz and colleagues (2008), the results support Broda’s (1971) hypothesis that individuals served as living impersonators of the deity to whom they were sacrificed. In this case, males were used for their ability to personify Ehecatl-Quetzalcoatl, the Aztec god of wind and rain, to whom the temple was dedicated.

A later study by Morales-Arce and colleagues (2019) also examined material from sacrificed subadults at Tlatelolco. Molecular sex data was obtained using a PCR based approach amplifying the amelogenin locus in addition to whole genome sequencing. Contrasting with the earlier study, Morales-Arce and colleagues identified 10 females and two males in their successful samples.

With results available from only these two studies and with sample sizes fairly limited, we urge caution in the interpretation of these data. Nevertheless, the results are intriguing for what they may suggest. While the sex of the deceased is different between the two studies of sacrificed subadults from Tlatelolco, this in itself is important. The male sex of the skeletons tested by De la Cruz et al. (2008) came from a single temple dedicated to the rain god Tlaloc. The authors suggest that victims were chosen to match this deity's sex. Morales-Arce and colleagues (2019) suggest that the predominately female composition of their sample may reflect different rituals or even positions in the calendar.

Our study reinforces the possibility that, in the Maya context, patterns of recruitment of subadults were based on sex. This pattern was unsuspected and prior to the advent of paleogenomics, there was no way to investigate the possibility. If the pattern is confirmed in future studies, it may provide insights into the gendered nature of particular temples, spaces, or deities involved.

The question of the sex distribution of adults has profound implications for the interpretation. The sacrifice of young male war captives has been a convenient corollary of the emphasis of warfare in the Maya collapse. Human sacrifice became part of the state program of aggression and intimidation. The focus on males was supported by the recovery of 72 sacrificed warriors from the Pyramid of the Plumed Serpent at Teotihuacan. Four of the warriors wore between seven and 11 human maxillae as trophies. Dental measurements suggest that they also had belonged to males (Spence and Pereira, 2007). However, this model had not been tested against archaeological data from the Maya lowlands.

The Cenote of Sacrifice is the one truly large sacrificial assemblage in the Maya lowlands. Hooten's (1940) analysis of the skeletal remains recovered by Thompson found an almost equal number of male and female remains (Hooten, 1940: 272). Our MNI estimation of seven males and five females is in accord with the sex ratios of the sample recovered from the Sacred Cenote, suggesting females form a significant component. The fragmentary number (10 male, 22 female) suggest that more women were present than men.

The inclusion of women in sacrificial assemblages at Chichen Itza and MTC would bring the warfare model into question. The model is further contradicted by the subadults found at both sites. These findings suggest that the Maya human sacrificial practices are complex and may interplay with the issues of gender in Maya social organization. It also suggests that the Cenote of Sacrifice sample with its mixed sex ratio may be representative of Maya sacrificial practices.

Our analysis of the sex ratio is further complicated with the results of the paleogenomic testing where eight of the nine individuals are female. The discrepancies between paleogenomic and osteological results probably reflect the difficulty assessing characteristics on a population with unknown or partially known expression of sexual dimorphism. All results undoubtedly reflect distortions based on the small sample size. We suspect that the "truth" about the MTC sex ratio lies somewhere in the middle, but this interpretation would mean that women made up more than half of the adults and possibly also of the subadults.

The MTC samples cover a wide age range. Our adult female samples seem to cluster in very young and middle age ranges. These two ranges are particularly interesting because they correspond to a widely recognized division of Mesoamerican female deities into a young and an old aspect (Thompson, 1939), noted in both ethnohistory and iconography. In the Maya area, accounts also mention the sacrifice of women who fall into these two age ranges. For example, in his *Relación*, Tomas Lopez Medel outlines the sacrifice of a virgin (Tozzer, 1944:223) in the petitioning of rain as well as an older woman in another ceremony (Tozzer, 1944:117). In Central Mexico, colonial sources mention victims impersonating the deity to whom they are sacrificed. With female deities, the sacrificed individual must match the deity in age as well as sex. When discussing the Great Feast of the Lords, Duran (1971:212) describes the sacrifice of a female slave, dressed as the stone idol in white with a white mantle. She received honors and courtly attentions as the goddess herself would have received. For the Feast for Hueypachtli (Feast of Flowers), another woman dressed in the guise of the goddess Xochiquetzali (Duran, 1971: 244) represented the goddess alive. She was sacrificed and flayed; then a man donned her skin and all her finery. A similar practice is also described for the feast in honor of Toci, Mother of the Gods and Heart of the Earth, where the female victim was usually around forty years of age (Duran, 1971:229). Duran (1971: 213-214; 250) also discusses the fact that the sacrifice of male captives and children were made to the woman representing the goddess in the ceremonies mentioned above. In one case this included the sacrifice of two children and in another four men. This could potentially provide insight into the males and children recovered from the Cenote of Sacrifice and at MTC.

While the MTC sample is small, the limited age distribution data suggest that females may have played roles as deity impersonators at the time of their deaths. Like rituals at Tenochtitlan, rituals carried out at MTC would have been sponsored by the polity itself and thus reflected elite level liturgy. Their selection of children, men, young women and older women as sacrificial victims probably closely aligned to the deity and the reasons for the solicitation.

The implications of addressing these questions victim demography through a combination of osteological and paleogenetic approaches profoundly change the discourse on sacrifice. We can move away from seeing sacrifice as an adjunct to warfare and towards a reconsideration of its role in ritual. Brady and Prufer (2005) have made a case for Maya religion being terrestrial in focus. Guiteras-Holmes (1961: 203) states explicitly that, for the Maya, *Earth* is both the supreme power and female. One would expect, therefore, that this female element was the focus of sacrifice and it underscores the significance of women in sacrifice.

5.6 Conclusions

This paper examines the Midnight Terror Cave skeletal assemblage with traditional anthroposcopic methods and paleogenomic analysis to advance our understanding of ancient Maya human sacrifice. While the anthroposcopic results show slightly more males than females, the raw count of sexed elements and the paleogenomic testing detected a strong preference for females, suggesting that there are more women than men in the assemblage. When combined with large number of subadults and the results from the Cenote of Sacrifice, it is clear that we are not dealing with an assemblage dominated by young males as suggested by the warfare hypothesis.

The paleogenomic determination that all four subadult pelves for which sex could be assigned were female is somewhat unexpected. This result suggests that preferences for one sex over another may have played a role in recruitment of sacrificial victims.

With the adult females, two age categories are represented, a young and an old aspect. This matches the two categories of female deities that Thompson notes in iconography. Ethnohistoric sources make clear that age and sex of victims were matched with the deities that they were impersonating in elite ritual performances. This determination allows us to outline a possible role in a ritual drama that the victims performed in at the time of their death.

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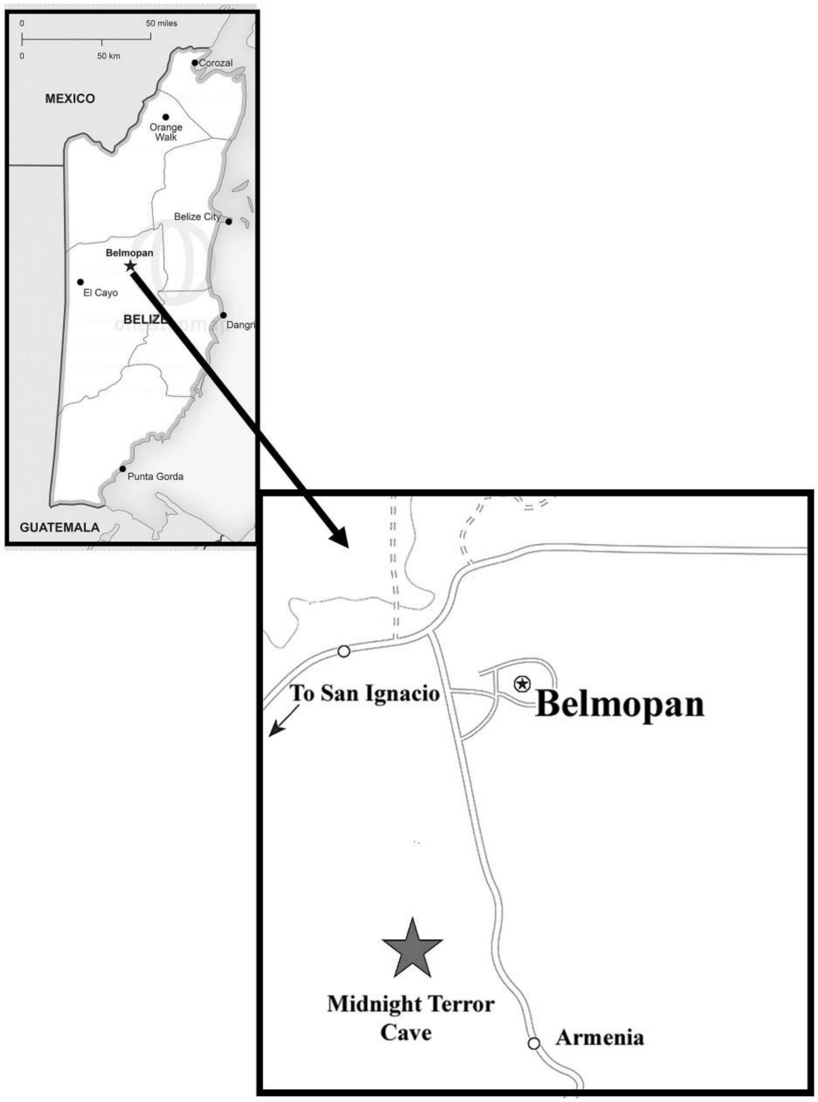


Figure 12. Map of Belize with inset showing the location of Midnight Terror Cave.

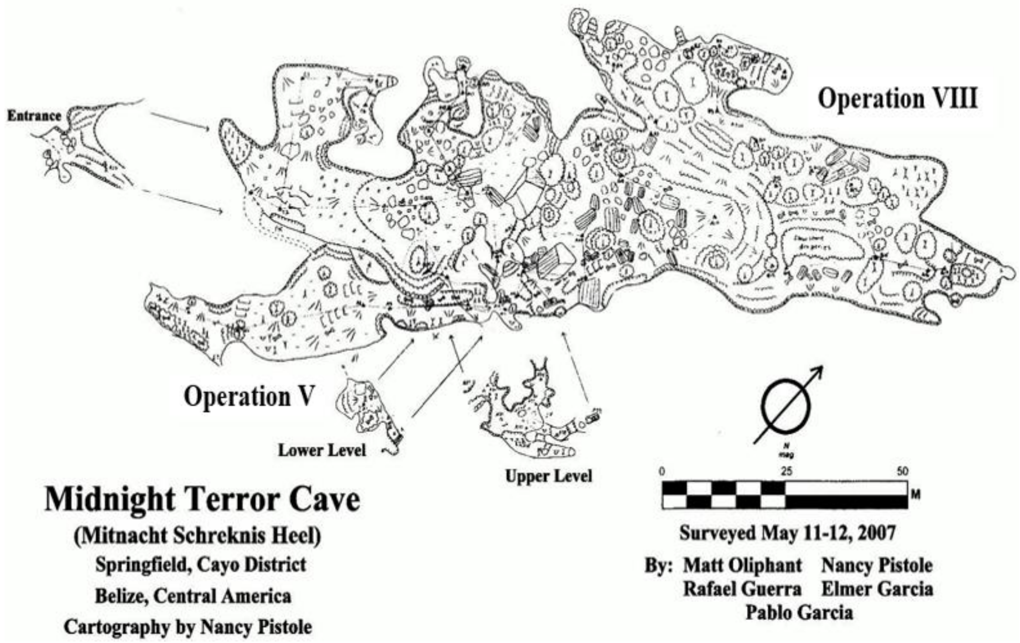


Figure 13. Map of MTC showing Operation V and Operation VIII.

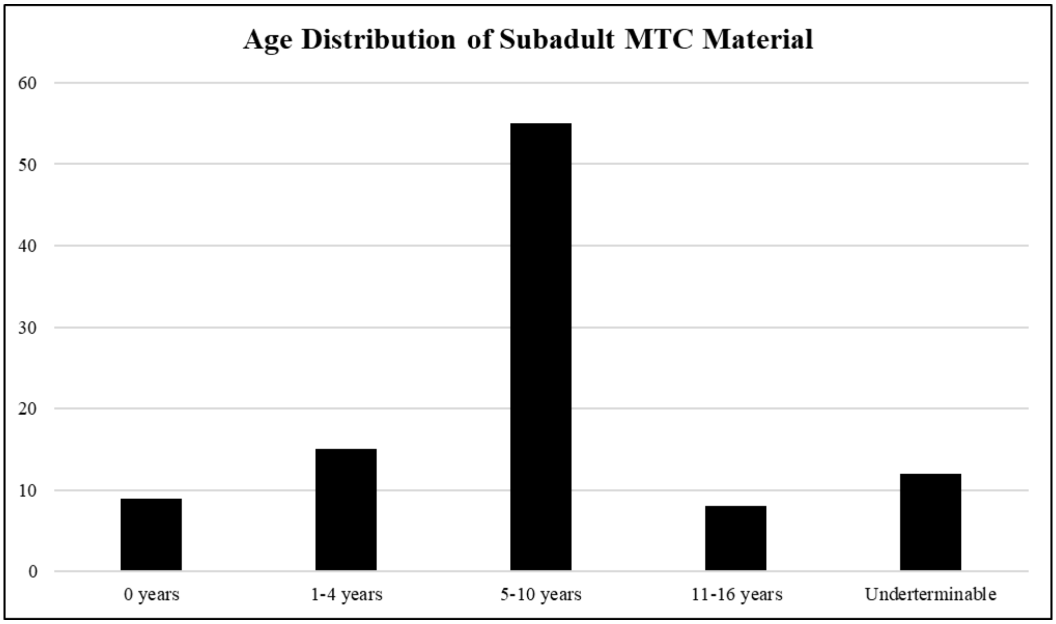


Figure 14. Subadult age distribution at MTC.

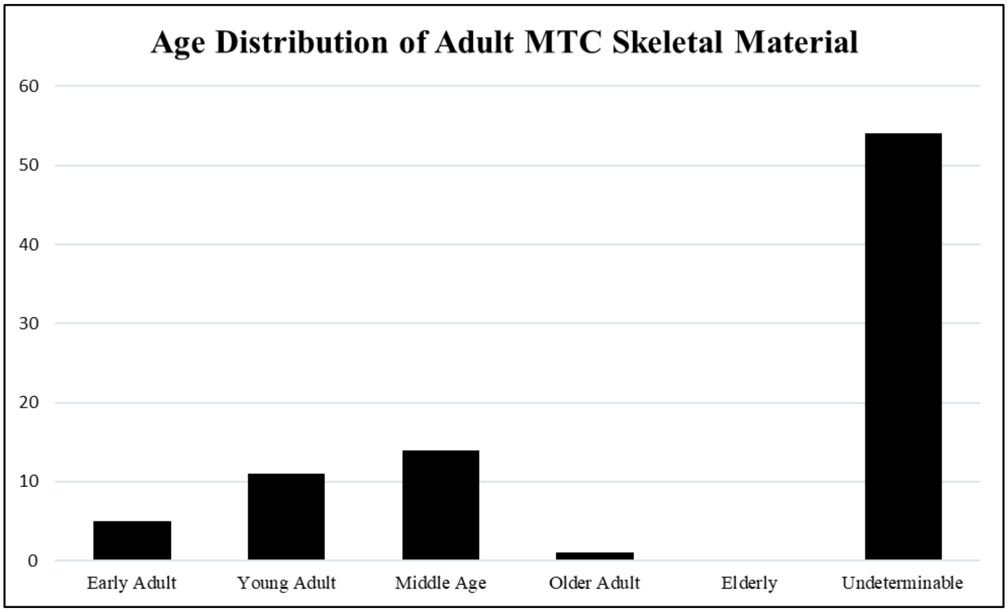


Figure 15. Age Distribution of adult remains at MTC.

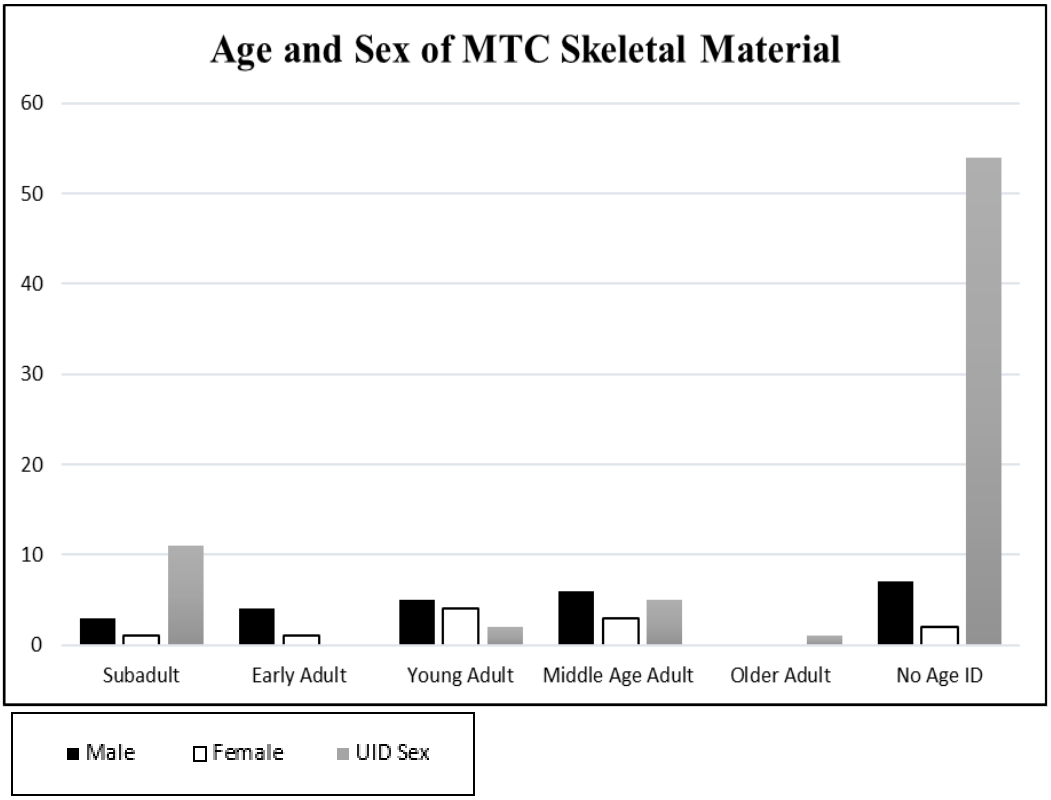
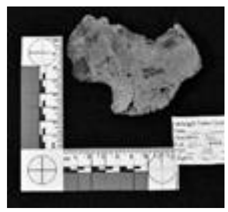
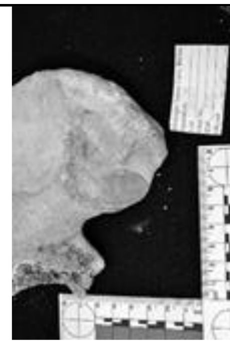
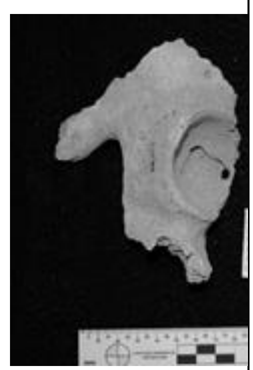


Figure 16. Age Distribution by Sex of the MTC skeletal material.

Table 3. Criteria used to estimate age categories. Where possible, photographic examples of MTC material included illustrating features aligning to the appropriate category.

	Epiphyses	Pubic Symphysis	Auricular Surface	Acetabulum
Subadult (0-late teens)	unfused	Billowing, unfused	Transverse organization of the surface 	
Early Adult (late teens-early 20's)	Recent fusion of the iliac crest, partial fusion of ischium	Partial or recent fusion of the ventral arc	Smooth, transverse organization	no groove in the acetabulum, rounded shape, apex and fossa are clean 
Young Adult (early 20's-mid 30's)	Fusion of iliac crest and ischium	Shows lack of margins at superior or inferior border and billowing remains pronounced on the surface	Billowing still present, marked transverse organization, no porosity	No groove present, partially narrow shape, no apex activity, no outer edge activity, dense acetabular fossa porosity

				
Middle Age Adult (mid 20’s to mid 40’s)		Some old furrows, oval outline, separated pubic tubercle, Face smooth, no lipping. 	Some loss of billowing, surface is coarse and granular, some microporosity 	Groove present, narrow or rough shape, external rim porosity, some osteophytic activity, between ½-3/4 out edge activity, peripheral acetabular fossa activity and porosity 
Older Adult (mid 40’s to mid 60’s)	Significant lipping	Face completely rimmed, moderate ventral lipping, pronounced dorsally	Absence of billowing, irregular margins, little or no macroporosity, apical changes	Pronounced groove present, crested and rough rim, presence on spicules on apex, macroporosity with destruction

Elderly Adult (over 65 years of age)		Broadening of the pubic symphysial surface with marked porosity, pubic tubercle is a separate bony knob	Significant porosity, irregular marginals typical of joint changes, profuse osteophytes	Very pronounced groove present, very high crested rim, very much osteophytic activity, destruction on most of the fossa, bone proliferation

CHAPTER 6

Concluding Thoughts

This dissertation employs an interdisciplinary approach that combines archaeology, ethnohistory, osteology, and paleogenomics to examine the MTC human skeletal assemblage and the question of human sacrifice. The analysis of the MTC skeletal remains produced demographic data for the individuals interred in the cave. This resulted in scientific data used to examine recruitment practices for human sacrifice. In addition, this dissertation outlined the evidence for the association of human sacrifice with cave spaces and, specifically, the evidence of human sacrifice at MTC. This data can recast current debates (Berryman, 2007; Brady, 1989; Anda Alanís, 2007; Demarest et al., 1997; Schmidt and Schröder, 2016; Tiesler, 2005; Wrobel et al., 2014) on human sacrifice.

Anthropological discussions of human sacrifice focus primarily on the practice as a religious act, concerned with ritual and associated beliefs (Bataille, 1991; Davies, 1981; Durkheim, 1965; Evans-Pritchard, 1954; Frazer, 1911; Girard, 1977; Hubert and Mauss, 1964; Robertson Smith, 1894; Spencer, 1882; Tylor, 1871; Valeri, 1985; van Baal, 1976; van der Leeuw, 1986; Westermark, 1912). The focus on belief is mired in the emic justification for the practice. Such beliefs are well documented for Mesoamerica in ethnohistorical sources but are of only limited value to archaeologists working with the physical remains from an act.

This dissertation has rejected those approaches in noting that discussions of the political nature of sacrifice are largely absent. As a political act, sacrifice asserts the state's right to take human life while deflecting possible resistance by cloaking it within a religious ritual. Sacrifices performed by individuals in political offices such as priests reinforce and legitimize power structures under the guise of reviving or sustaining the existing social order (Lincoln, 1991). Lincoln notes that sacrifice is largely asymmetrical, benefiting one social group through the destruction of another. As a result, it is important to consider the way in which sacrifice is tied to the larger social and political landscape. This dissertation ignores the emic rationalization of sacrifice to construct an innovative model utilizing elements from ritual violence and performance theory to demonstrate that public performance is an essential component in legitimizing the act of human sacrifice.

In order to examine the link between polity power structures and sacrifice, this research focused on understanding how individuals were obtained for human sacrifice. Identifying patterns in sex, age, residency, and possible lineage affiliation may suggest the position of one group of people over another. With the addition of genomic data, it is possible to assess how MTC individuals fit into the larger Mesoamerican and more specifically, Maya population structure.

6.1 Research Questions

Having chosen to focus on the identification and recruitment of victims, a number of specific research questions were developed that would serve to operationalize the inquiry.

Question 1: Are individuals at MTC from the local settlement of Tipan Ch'en Uitz (TCU)?

Question 2: Does the MTC population show preferential selection based on sex?

Question 3: Were men, woman, and children recruited from different populations? It was felt that the genetic data derived from the MTC assemblage, could clarify our very murky understanding of the demographics of sacrifice, crucial to any attempt to move beyond the current understanding.

The first question, are individuals at MTC from the local settlement of Tipan Ch’en Uitz (TCU), was designed to answer the very basic question of whether recruitment occurred within a polity or from outside of the borders of the society. Theories of human sacrifice rarely consider the form of recruitment as an inherently political act that impacts a polity’s own population or that of its neighbors. Determining the local or nonlocal affiliation of the MTC population is critical to understanding how individuals were selected. With this information, new insights into the practice of human sacrifice by the ancient Maya would be elucidated.

Circumstances prevented this question from being examined in this dissertation. After investigation and conversation with Gabriel Wrobel, the director of the TCU project, it was found that no human skeletal remains have been recovered from TCU. This is largely due to limited excavation of the site. It is hypothesized that human remains may be present underneath household floors, a common practice by the ancient Maya. It is also possible that human remains might be interred at rockshelters as this also seems to be a practice by the ancient Maya in this part of Belize. The attempt to obtain skeletal remains from Cave Branch Rockshelters (CBR), a burial site located approximately 20 kilometers from TCU, were unsuccessful (Bonor, 1995, 1997; Bonor and Glassman, 1999; Bonor and Martinez Klenn, 1995; Glassman and Bonor Villarejo, 2005; Wrobel et al., 2007, 2010, 2017). This remains an important question in addressing how sacrificed individuals were recruited. By understanding how the MTC skeletal assemblage compares genetically to population of TCU or CBR, it will be possible to see if MTC individuals are more or less likely to be from the local settlement population or come from elsewhere.

The second question, does the MTC population show preferential selection based on sex, became central early in the osteological analysis as females were clearly present in the assemblage. This ran counter to current thinking that assumed that victims would be predominantly young males captured in battle. Warfare, then, provided a simple and straightforward explanation to the questions of both internal or external origins and sex distribution. The idea's only shortcoming is that it had never been verified with empirical data. The MTC assemblage suggested that the warfare hypothesis has to be rethought.

This question of the selection of sacrificed individuals based on sex has been directly and successfully addressed in Chapter 4. It is important to clarify that "sex" and "gender" are related but not equivalent and in this study, we are addressing sex, not gender. Analysis demonstrates the presence of both males and females and hints at female preference. These data, at minimum, illustrate that MTC is not dominated by young males as suggested in the warfare model (Demarest et al., 1997). Additionally, paleogenomic analysis identified a number of subadults as female suggesting that selection by sex was far more pervasive than ever previously suggested, extending even to the selection of children.

The final question, were men, woman, and children recruited from different populations, was an outgrowth of documenting large numbers of women and Prout and Brady's (2018) work that drew attention to the large percentage of subadults in the MTC collection. This underscored the anthropological axiom that all societies divide labor by age as sex. Were individuals in the three different statuses recruited differently? Such complexity had not been previously injected into the question of sacrifice.

On-going analysis will address the amount of genetic variability in the male, female, and subadult samples to determine if they were recruited from the same or different populations. Obtaining data to answer this question has taken the greatest amount of time, largely due to getting the number of samples necessary to address the question; hundreds of MTC samples were tested. Many samples did not yield usable DNA due the highly degraded condition of the material. The strategy followed sampling bone and teeth from each Operation within the cave and of both subadult and adult remains. It was found that teeth were best for obtaining DNA data and make up the largest portion of the total sample. The difficulty in using the MTC teeth is that a majority of teeth were recovered unassociated with skull bones or fragments. Though we did sample teeth from different areas of the cave, without their association with bone, there was no way to be sure we sampled different individuals until the results from the genetic testing were revealed.

After extracting the DNA, creating libraries, and amplifying the libraries, the samples were sent to Jena, Germany for 1240K SNP genome testing. Currently, the results are being analyzed.

6.2 Significance of Chapter 2

At the heart of any bioarchaeological study on sacrifice is the physical evidence that demonstrates the remains are related to sacrifice. This chapter takes a novel approach by forcing the consideration of space and burial type into conversations concerning sacrifice. A noticeable pattern has emerged in that osteologists unconvinced an assemblage is related to sacrifice due to the lacking evidence of physical violence then *assume* some form of burial. Instead, this study puts the onus on both sides by demanding that the form of burial be specified so that the characteristics of that form can be elaborated and compared to the assemblage in question.

As was apparent in Chapter 2, this placed a great deal of emphasis on the archaeological context of the deposition of the material. This began with examining cave use by the Maya and considering how the MTC remains fit the larger picture of Maya cave archaeology. The point emphasized here is that contrary to many discussions for the practice of human sacrifice, the archaeological context, in addition to the physical remains, must be included as evidence for determining sacrificial from non-sacrificial assemblages. Cutmarks, alone, cannot be the deciding factor in proving the occurrence of human sacrifice. Chapter 2 reviewed the evidence for burial and sacrifice in Maya archaeology to demonstrate clearly that the MTC assemblage reflected the practice of human sacrifice rather than burial. Furthermore, the fact that most cave remains are surface depositions suggests that in most cases in the Maya area they will be related to sacrifice.

6.3 Significance of Chapter 3

The re-examination of Kieffer's (2015) claim that social outcasts were sacrificed at MTC based on a physical deformity caused by Klippel-Feil Syndrome showed that a critical contention in her argument was incorrect. Analysis demonstrated that Kieffer's (2015) assertion that a number of bones exhibiting pathology all belonged to the same individual based on there being only a single adult individual, was not supported by our findings. Paleogenomics verified that the fused vertebra supposedly the result of Klippel-Feil Syndrome was from a different individual than one of the skull bones.

The osteological portion of the analysis demonstrated repeatedly that there was a minimum of two adult individuals. When this was combined with the multiple subadult individuals, it provided a graphic picture of the degree of commingling in a single lot (VI-02). This is an important point that was discussed in Chapter 2.

Theoretically, Kieffer (2015) applies a "scapegoat" model to suggest that two individuals may have been selected for sacrifice based on their physical handicaps that allows the author to characterize them as "social outcasts." In the absence of independently derived data, the application of a "scapegoat" model becomes an exercise in circular reasoning in which victims' status as social outsiders is simply assumed by virtue of their having been sacrificed. The strength of Kieffer's proposal is that she specifies the physical features used in applying the theoretical model. Nevertheless, problems lay in the knowledge requirements about the sacrificial victims in a context where the mortuary treatment appears to be designed to strip victims of personhood.

Results outlined in Verdugo et al. (2016) reinforce Prout's (2015) contention that the individual was most likely asymptomatic and so would not have been sacrificed as "social outcasts" based on a physical deformity. Scott (2015) shows that the physically deformed held high status in Pre-Columbian Mesoamerican society, so could not be considered "social outcasts" and presents evidence that such individuals were sacrificed only under extraordinary circumstances.

6.4 Significance of Chapter 4

It should be noted that the peer-review process substantially changed the direction of this paper to emphasize the paleogenomic results and away from social data that had implications for the question of sacrifice. The application of paleogenomics for sex determination indicates that that more than 60% of our sample is female, contrasting with models that predict that most victims are males conscripted as war captives. Our results also hinted at a link between modification style and kinship.

Analysis of dental modification practices at MTC is also related to the question of sacrifice because the practice is generally seen as an elite status marker (Becker, 1973; Labajo González et al., 2007; Romero, 1958; Smith, 1972). Williams and White (2006), however, argue against this idea noting the lack of evidence illustrating a connection between dental modification and socioeconomic status. The lack of caries and other dental defects at MTC, however, did indeed suggest the dental modification was a high-status marker.

The large size of the MTC assemblage is ideal for exploring the implications for sacrifice. With 30% of the incisors showing modification of some form, it could be claimed that one-third of the MTC population held elite status. This seems highly unlikely. Scholars (Chase and Chase, 1992; Sharer and Traxler, 2006) generally agree that elites comprise no more than about 10% of the population in agrarian states. It has never been argued, however, that MTC represents a faithful cross section of Maya society. We have argued that the extensive modifications at MTC mark it as the principal ceremonial stage for TCU. As the scene for state level public ritual, MTC may have played host to the sacrifice of the most important individuals.

Additionally, when Maya inscriptions mention the sacrifice of high-status individuals, they are invariably adult males. The iconography of warfare also tends to depict captives as adult males. The MTC data suggests that these sources are reflecting the politics of representation which is not supported by the archaeological evidence. While the inscriptions and iconography depict sacrificed individuals as adult males, the skeletal collection contains a significant percentage of women and almost half the individuals (MNI) are children (Prout and Brady, 2018). The archaeological evidence, therefore, provides the truest picture of what occurred. The high incidence of dental modification and low incidence of enamel hypoplasia and dental caries suggests the MTC population held elevated status but was not confined to males.

Our data suggesting that individuals were selected for sacrifice at MTC based on their high social status has additional theoretical implications. Current notions of sacrifice are heavily grounded in Marxian thinking, so scholars tend to see sacrifice as an honor gifted on the lower classes. While it is possible that MTC's close connection to TCU means that its population is not representative of sacrifice in general, it stands as a warning against allowing theory to dictate interpretations (allowing interpretations to simply recapitulate theory).

6.5 Significance of Chapter 5

Chapter 5 also examines sex in addition to age. Pelvic data demonstrates the presences of adult and subadult males and females in the MTC skeletal assemblage. The subadults ranged in age from less than a year old to sixteen years of age. Interestingly, age range of subadults peaks at 5-10 years, when mortality rates normally drop.

The adult age range is noteworthy because differences are seen between males and females. For adult males, age falls primarily into the Young (early 20's- mid 30's) and Middle Adult (mid 20's to mid 40's) ranges. Adult females appear Early Adult (late teens-early 20's**)**, (4) Young Adult (early 20's- mid 30's), and Middle Adult (mid 20's to mid 40's) age ranges. Data suggests that females cluster in the very young and middle age ranges. Interestingly, these ranges correspond to widely recognized young and old aspect of Mesoamerican female deities (Thompson, 1939) identified ethnohistorically and iconographically. This suggests the selection of women based on their alignment to the deity to whom they were sacrificed.

An unexpected result of the paleogenomic analysis was that all four of the subadult pelvises tested were female. This is the first evidence to suggest that Maya subadult victims may have been selected based on sex. Two studies of Aztec subadult sacrifices found that victims were predominantly of one sex, in one case male (De la Cruz et al., 2008) and in the other case female (Morales-Arce, 2019). These data suggest that a Mesoamerica-wide pattern may have existed of selecting victims for sacrifice based on the sex of the deity to whom they were being offered or perhaps even the perceived sex of the place in which they were being offered.

This dissertation sought to test theoretical discussions of human sacrifice by producing scientific data that would provide new insights into the practice. Interestingly, although the emphasis was on determining age and sex, this research was able to speak to the ritual of sacrifice when sex and age data strongly indicated that women may have been playing roles as deity impersonators. Although this research has eschewed a focus on emic explanations for sacrifice, nevertheless, our new insights provide a window into the form and meaning of these ritual dramas.

At an even higher level, our results detailing the prevalence of women in Maya sacrifice and their role as impersonators of female deities, strongly supports the importance of a cosmology focused on the sacred *Earth,* which is clearly gendered female in Mesoamerica. This is clearly reflected in the importance of the pilgrimage center on Cozumel Island dedicated to the goddess Ix Chel (Patel, 2005). This is replicated across Mesoamerica. H.B. Nicholson says, “of the many deities portrayed by the surviving [Aztec] stone images, more probably represent the fertility goddess than any other single supernatural” (quoted in Markman and Markman, 1994: 185)

6.6 Methodological Take-Aways

This research also yielded a number of unexpected insights unrelated to the main research questions. Throughout the course of this project, paleogenomics proved a useful tool for supplementing the osteological data. Given the commingled and high fragmented state of the MTC bones, paleogenomics offered a means with which to obtain demographic data, such as sex, necessary for answering questions about sacrifice. Additionally, this research demonstrated that use of genetics for distinguishing between commingled individuals. This was particularly evident in the research outlined in Chapter 2. The reanalysis of MTC remains provided a trial run for applying paleogenetic techniques as integral to osteological analysis in order to obtain more detailed information on the MTC assemblage. From a methodological perspective, paleogenomics cut through the complexity and diversity within the bones examined by Kieffer (2015) who had constructed a plausible but untestable context for suggesting that all of the pathological elements definitively showed that more than one individual was present. The successful application of this approach in this early MTC study laid the groundwork for the remaining dissertation research.

6.7 New Directions for MTC

As previously noted, Question 1 regarding the link between TCU and MTC remains a crucial question for addressing sacrifice. As archaeological investigation at

TCU progresses, it may one day be possible to assess the genetic variability for each site and between the sites. Obtaining this data would allow for the examination of whether individuals sacrificed at MTC were local or non-local to TCU.

One way to begin addressing the local vs non-local issue is to compare the genetic variability recovered from a cave not associated with the practice of human sacrifice to the genetic variability determined with MTC individuals. This is on-going research at the UCSC Human Paleogenomics Laboratory. Skeletal remains recovered from caves at Dos Pilas, Guatemala were sampled in order to compare with MTC data. According to Minjares (2003), these remains show no evidence of human sacrifice. Difference in the amount of variation may suggest different interment practices. Less genetic variability between sample suggests that the individuals come from the same place. Conversely, greater genetic variability between samples may suggest individuals come from different locations. Again, subadult and adult teeth were sampled from different caves and different areas which each cave.

Another opportunity for MTC research involves dental cementum incremental analysis (DCIA). The approach provides data for an individual’s life history based on dental cementum bands (Wedel and Wescott, 2016). The reduction or increase in calcium consumption produces opaque or black zones under polarized light on teeth. According to Wedel and colleagues (2012), collagen fibers appear dark in winter due to the arrest in cementum increment as opposed to the summer which college fibers appear translucent or light. The pair of these two growth increment patterns represents one year in a person’s life (Wedel and Wescott, 2016; 136). Examining

MTC teeth using DCIA should yield insight into whether people were sacrificed during the same season each year, or whether it varied depending of demographic data. Assessing the seasonality of death would test whether the practice was more common during the winter (dry season) or summer (wet season). My initial expectation is that deaths may cluster around the end of the dry season. In the Maya area today rituals for rain and a good harvest are conducted in caves and mountain tops on *Dia de la Cruz* (May 3rd). The rituals are thought to have Pre-Columbian roots. Data could test hypotheses linking human sacrifice to the drought cult (Moyes et al., 2009).

6.8 Final Thoughts

There has been a growing awareness over the last several decades that caves are powerful landmarks that elites often attempt to appropriate. García-Zambrano (1994:218) states explicitly, "These cavities, when ritually dedicated to the divinities, became the pulsating heart of the new town, providing the cosmogonic referents that legitimized the settlers' rights for occupying that space and for the ruler's authority over that site." This is well illustrated in Olmec iconographic programs that link rulers to caves. An early example is La Venta Altar 4 where the central motif of this throne is an individual emerging from a cave (Grove, 1973). A later example is the Chenes monster mask facades which symbolically transform a ruler's palace into a cave.

Given this strong tie between caves and legitimacy, political centers were often associated with caves. These relationships have been documented at sites in

Belize, Guatemala, and Mexico (Brady and Ashmore, 1999; Halperin, 2005; Thompson, 1939). The deliberate nature of such placements is most convincingly demonstrated with the construction of man-made caves in areas where natural caves do not occur as at the sites of La Lagunita (Ichon and Arnauld, 1985), Utatlan (Brady, 1991), Acatzingo Viejo (Aguilar et al., 2005) and Teotihuacan (Heyden, 1975; Millon, 1981). The simultaneous religious and political nature of prominent cave features is reflected in evidence of desecration of caves following military defeats (Brady and Colas, 2005; Helmke and Brady, 2014).

This research has sought to analyze political power through the institution of human sacrifice. The final and critical component necessary for drawing these concepts together is performance. This is a logical consideration given the important role of performance within violence theory (Riches, 1986; Schmidt and Schroder, 2001). Numerous scholars emphasize "the power and effect of theatrical performance [for defining] the political reality and social relations behind which the real mechanism of power works" (Coben and Inomata, 2006: 28). In fact, it has been argued that performance is a critical part of politics given that it is impossible to have social relations without symbolic acts and social ritual (Bell, 1992; Kertzer, 1988; Douglas, 1966; Geertz, 1980).

As a religious ritual practiced in communal space, sacrifice is obviously a public performance but, surprisingly, performance has received scant attention from ritual theory. Inomata and Coben (2006), however, argue that the public performance of theatrical events served as a tool in Classic Maya politics that facilitated community cohesion while marketing the agenda of those in power. Such events occurred in varying spatial scales ranging from large plazas to smaller residential complexes to sacred landmarks such as caves (Inomata, 2006: 810). In shifting the approach to sacrifice to that of violence, performance becomes a central element. Thus, the performative aspects of sacrifice and violence effectively map power relations in a religious context where they are less likely to be contested.

Analysis of the MTC skeletal assemblage has clarified the demographics of human sacrifice in terms of age and sex. While the possibility that warfare offered an avenue for obtaining individuals for sacrifice remains, MTC shows clear differences than the male war captives hypothesis. The data illustrating the large presence of females and subadults offers a new perspective of human sacrifice which identifies sex as vital to the constitution of human sacrifice. This point is compounded when considering the sex of children may have also been a component of the ritual. These new data provide a glimpse of the emic understanding of sacrifice for the Maya not seen before. Now it becomes possible to see the intentional selection of certain individuals based on their ability to impersonate deities.

While this inquiry began with a focus on the biological and archaeological evidence at MTC, it now allows us to glimpse something of the politics and religion surrounding MTC. It is clear that ritual occurring within MTC was about show and drama. The enormous effort necessary to construct pathways and terraces deep within the cave provided the infrastructure for performance. The outline of the performance is evident in all classes of artifacts, not simply the human remains. Ceramic vessels undoubtedly holding a variety of offerings are concentrated around the plaza in the entrance to the cave while human sacrifice occurred in Operation VIII overlooking the plaza in Operation VII. It also occurred on the platform and altar in Operation V.

Recently an analysis of the MTC faunal assemblage drew attention to the remains of brilliant blue or green parrot fish on the plaza in the cave entrance. The analysis makes a case that the fish were brought alive some 45 kilometers from the coast (Brady et al., 2019). The authors emphasize the great effort and expense lavished on elite ritual. Interestingly, the discovery of the fish remains on the plaza in the entrance presents evidence that this offering was conceptually different for the Maya from human sacrifice occurring in the interior of the cave. The documentation of differing ceremonies along a constructed circuit, bespeaks the performance of a lengthy and sophisticated ritual drama. Finally, the use of elites in sacrifice provides eloquent testimony to the very political nature of these rituals.

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