Journal of Archaeological Science 115 (2020) 105096

Contents lists available at ScienceDirect

Journal of Archaeological Science

journal homepage: http://www.elsevier.com/locate/jas

An investigation of ancient Maya intentional dental modification practices at Midnight Terror Cave using anthroposcopic and paleogenomic methods

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ARTICLE INFO

Keywords:
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.

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 et al., 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; López Olivares, 1997; 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.

1.1. Background

Intentional dental modification is found primarily in the maxillary and mandibular incisors and occasionally the canine (Evans, 1973;

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https://doi.org/10.1016/j.jas.2020.105096
Received 2 August 2019; Received in revised form 19 December 2019; Accepted 27 January 2020
Available online 7 February 2020

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) system, 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

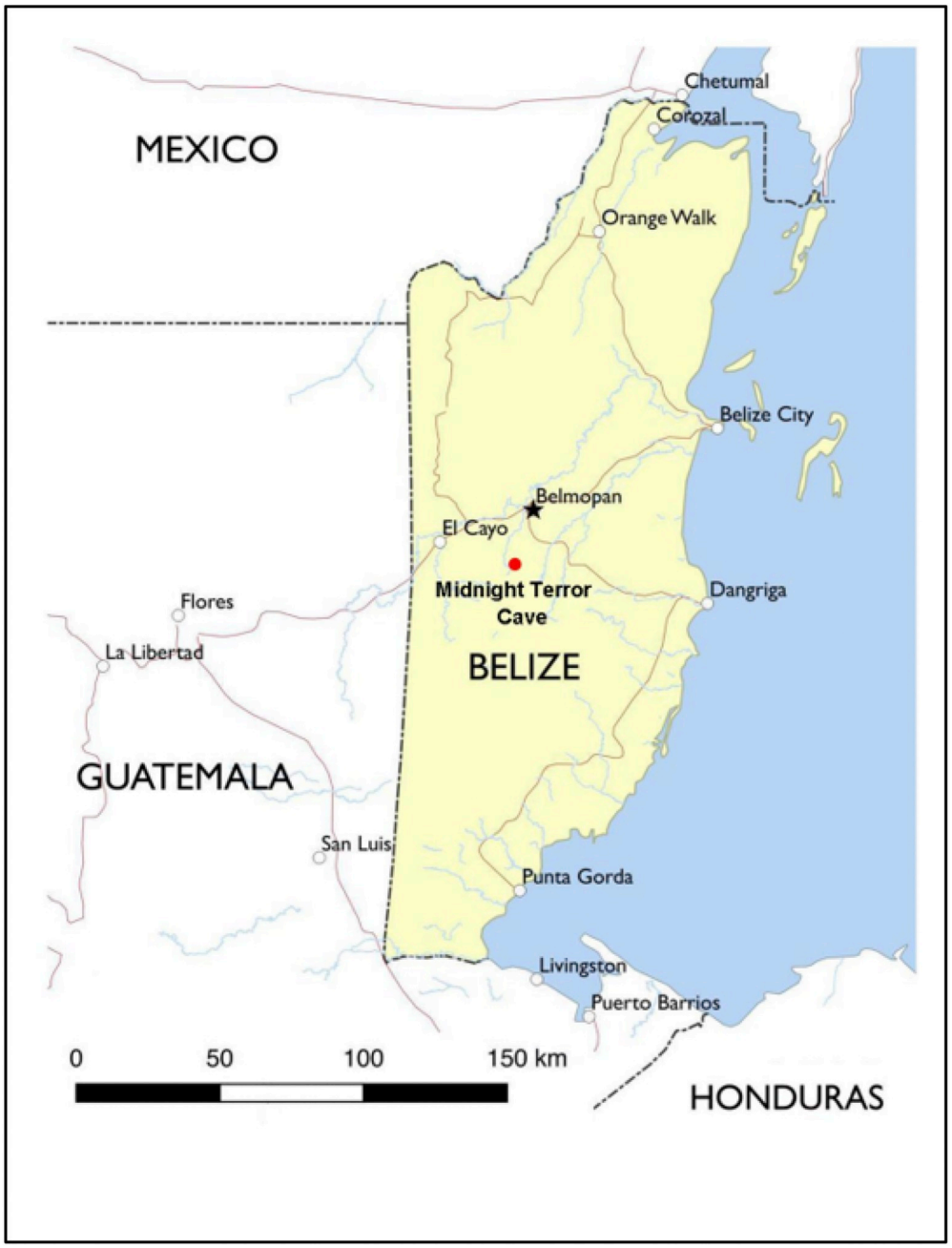


Fig. 1. Location of midnight terror cave, Belize.

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; López Olivares, 1997; Tiesler, 2001) 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.

1.2. Midnight Terror Cave

Midnight Terror Cave, located eight miles south of Belmopan in the Cayo District of Belize, was investigated from 2008 to 2010 as part of the Western Belize Regional Cave Project under the direction of Dr. Jaime Awe (Fig. 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.].

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, 2018). 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.

2. Material and methods

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 comingled. 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 Fig. 2). It is in these areas of the cave where the large majority of skeletal material was deposited.

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 (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° (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

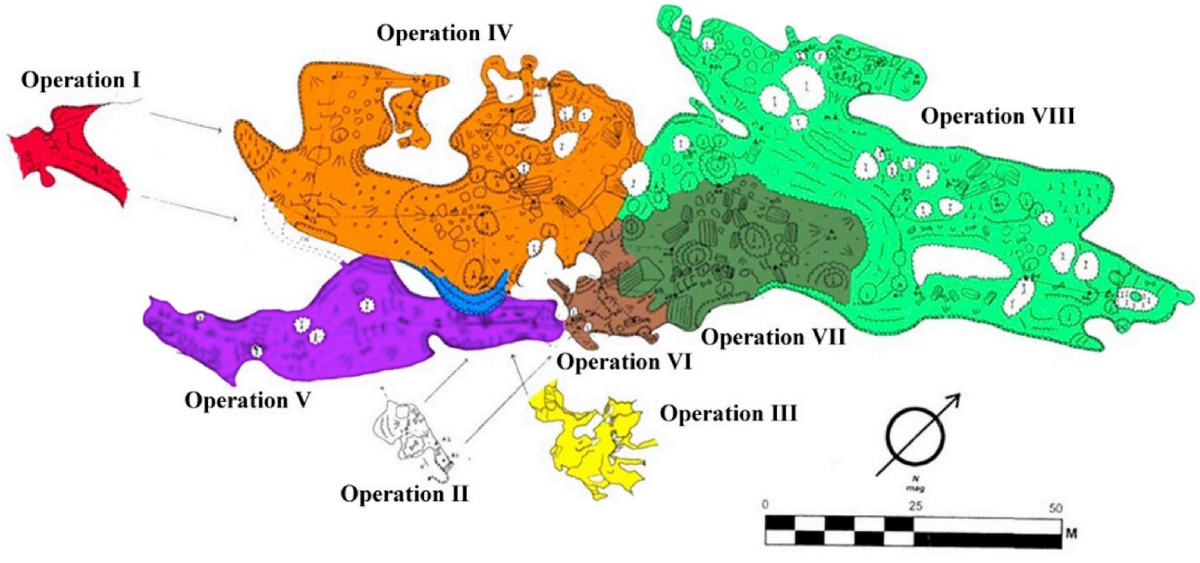


Fig. 2. Map of midnight terror cave.

Professional software analyses in order to ensure that a permanent model is still available (see https://data.mendeley.com/datasets for models). 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 h 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-min 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 and 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 2 × 75 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 (2 × 75 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., 2015). 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.

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. Fig. 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 Fig. 3). Two of the teeth could not be classified according to

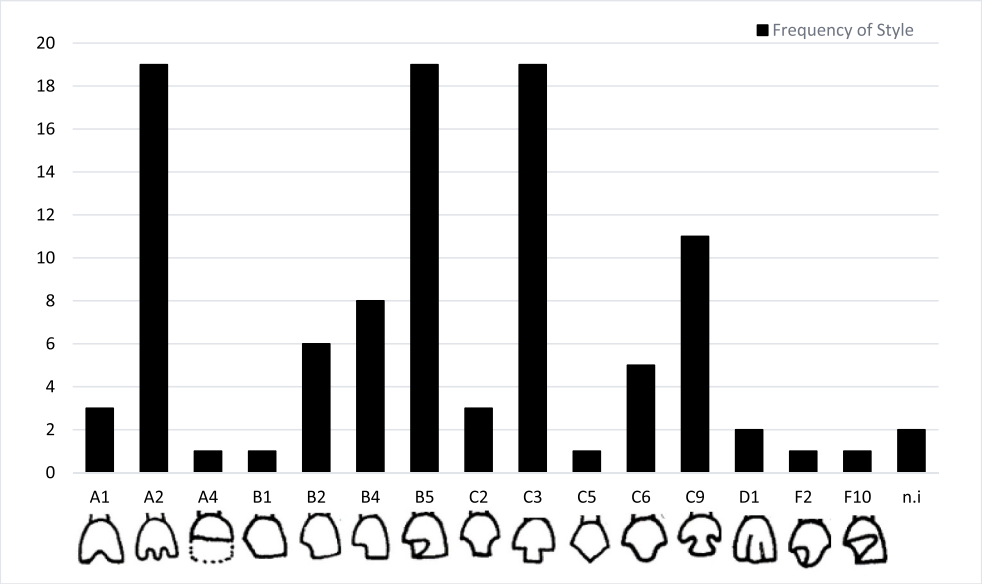


Fig. 3. Frequency of intentional dental modification style.

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.

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.

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. 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-Martín 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, Fis. 3–4; Teisler, 2000: 47; Dufoo Olivera et al., 2010: 100 Fis. 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.

4.1. Linking sex and intentional dental modification

There is little question that at least part of the explanation for

Table 1
Haplotype and sex determination paleogenetic results.

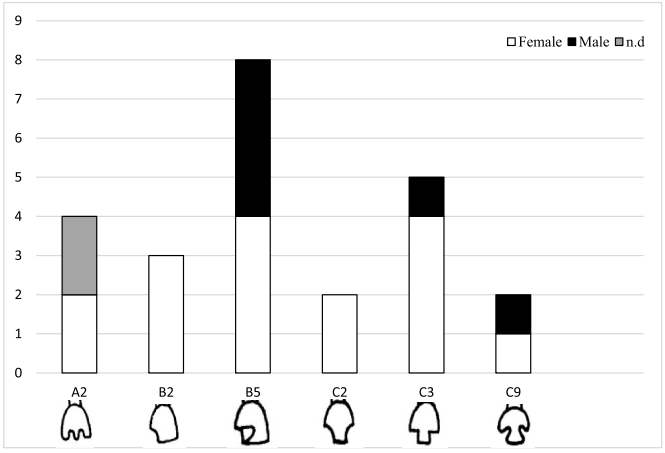
Sample	Modification Style	mt Haplotype	Shotgun Seq Sex ID	MtCapture Sex ID
V-01E-2003	B2	not analyzed	XX	not analyzed
V-01E-2004	A2	not enough data	XX	not enough data
V-01E-2005	C9	not analyzed	XY	not analyzed
V-01G-2001	C2	A2	XY	XY
V-03G-2001	C2	A2r	XY	XY
VI-01A-2001	C3	not analyzed	XX	not analyzed
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.

intentional dental modification is aesthetic so certain styles may be regionally or temporally popular. López Olivares (1997) 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

Table 2
Modification Style and Sex.



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 Gentry Steele, 1997; Williams and White, 2006; Saul and Saul, 1997; Scherer, 2018) also report finding the practice of intentional dental modification in both males and females in near equal numbers throughout the Maya area.

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.

4.2. 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 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.

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.

Acknowledgements

Research at Midnight Terror Cave was conducted with the permission and support of the Belizean Institute of Archaeology. Funding for this project was made possible by a generous grant from Lloyd Cotsen to California State University, Los Angeles. The senior author's Chancellor's Doctoral Incentive Program Mini-Grant funded DNA extraction and library preparation of the samples. The mitochondrial data outlined in this study is available at the National Center for Biotechnology Information under the accession numbers NCBI: MN848572 to MN848588.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jas.2020.105096.

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