Sex Identification of Children Sacrificed to the Ancient Aztec Rain Gods in Tlatelolco

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Recent excavations of Temple R, dedicated to the Aztec god of wind and rain, Ehecatl-Quetzalcoatl, at the archaeological site of Tlatelolco, Mexico City, recovered the sacrificial remains of 37 subadults and 6 adults. It is believed that this ceremonial complex was the site of an extraordinary ceremony carried out during the great drought and famine of AD 1454–57, a date consistent with the founding of this temple. The ages of 31 subadults and 1 adult were estimated by standard morphometrics analysis of the skeletal remains. Most of the subadults (66%) were children up to 3 years old. Through the extraction of ancient DNA from these remains, it was possible to use molecular techniques to determine the sex of the skeletal remains where in many cases (subadults and fragmentary remains) morphometrics analyses failed. Most, if not all, of these sacrificial victims were males. This remarkable gender bias is consistent with the notion that the victims chosen for sacrifice were a living impersonation of the god to whom they were offered.

The ritual sacrifice of children to the ancient Aztec gods of rain was documented by Spanish chroniclers in the sixteenth century (Benavente 1979; Durán 1984; Sahagún 1975). However, little archaeological evidence of this practice was available until recent discoveries were made in the Templo Mayor of Tenochtitlan (Román 1990) and in Tlatelolco (Guilliem 1999). These, the earliest and largest Aztec cities, were founded on small neighboring islets in Tezcoco Lake, and their ruins are located in what is now central Mexico City.

Tlatelolco's Temple R (see CA+ online supplement A), dedicated to the Aztec god of wind and rain Ehecatl-Quetzalcoatl, is contemporary with stage IV of Tlatelolco's Major Temple (AD 1428–67). Recent excavations in this temple recovered the skeletal remains of 37 subadults and 6 adults accompanied by many valuable objects; together, they provided evidence of a sacrificial offering made in a single ceremony and laid down with remarkable care and order (Guilliem 1999). Seventeen infants and 1 adult were buried in globular ceramic urns while the remainder were buried directly in the ground (fig. 1; table 1).

While morphometric analysis of human skeletal remains can provide a reliable estimate of the age at death of an adult individual, sex identification of subadult or fragmentary skeletons based on morphometric analysis is unreliable (Bass 1989; Brown 1998, 2001; Ubelaker 1974). Molecular techniques, in contrast, based on polymerase chain reaction (PCR) amplification of segments of the amelogenin genes, allow the reliable sex identification of subadult or fragmentary skeletons (Faerman et al. 1995; Götherstrom et al. 1997; Stone et al. 1996; Sullivan et al. 1993). However, PCR inhibitors (e.g., humic acids, fulvic acids, and tannins) coextracted with ancient DNA (aDNA) and/or the degradation of aDNA (Pääbo 1989) can cause incorrect sex identification because of PCR failure and allelic dropout. To reduce the rate of false sex identification, results should be independently confirmed through multiple DNA extractions by separate laboratories (Brown 2001; Lassen, Hummel, and Hermann 1996; Meyer et al. 2000; Schmidt, Hummel, and Hermann 2003). Independent replication is most effective when the replicating laboratory employs its own independent methods for extracting and analyzing aDNA (O'Rourke 2007).

Sex identification of the victims of Tlatelolco's sacrificial offering can contribute to a better understanding of these ceremonies. To this end, we screened portions of the amelogenin gene that exhibit length dimorphisms on the X and Y chromosomes to identify the sex of the skeletal remains. The screenings were carried out independently in two laboratories (Universidad Nacional Autónoma de México [UNAM] and the University of California, Davis [UCD]) from different bones, each laboratory using its own extraction and amplification techniques. In the subset of samples sent to UCD, we also screened two single nucleotide polymorphisms (SNPs) of the Y chromosome that define haplogroups Q-M242* and Q-M3* and the founding Amerindian mitochondrial DNA (mtDNA) lineages to authenticate the ethnic identity of the samples.

Materials and Methods

Samples

The skeletal sample consisted of 43 individuals, 37 subadults and 6 adults, found under the front platform of Temple R in the ceremonial center of Tlatelolco. Most of the subadult skeletons were complete or nearly complete; only four of them were fragmentary. However, only one adult skeleton was

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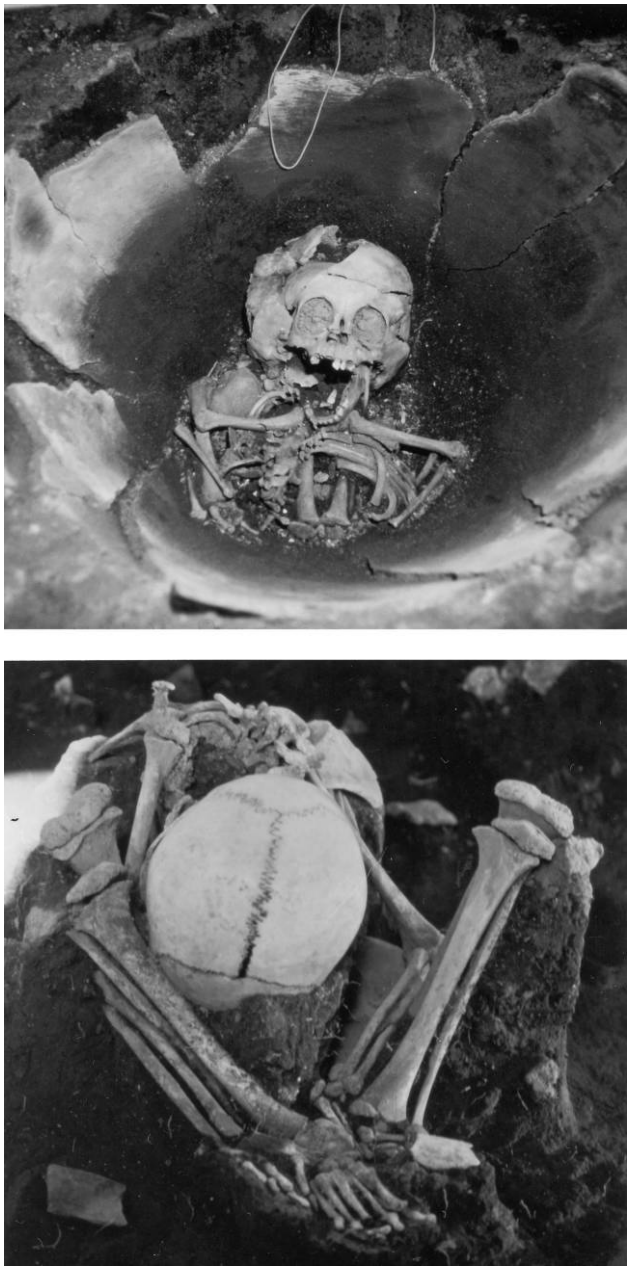

Figure 1. Infant individuals in situ during the archaeological excavations at the temple of Ehecatl-Quetzalcoatl. *Top*, two-year-old buried inside an urn. *Bottom*, six-year-old buried directly in the ground with a thin flat stone supporting his back.

nearly complete. A second adult skeleton consisted of a fragmented skull and all the vertebrae, while only chest bones (vertebrae and ribs) of the remaining four adults were present. The fragmentary state of the adult skeletons was probably due to intentional dismembering of the corpses (Guilliem 1999). The excavation was conducted during three seasons (1987–89) by a team of six archaeologists (five females, one male) under the direction of the archaeologists S. Guilliem and F. Hinojosa, both males; it was not possible to identify the relationship between a particular worker and the samples. After the excavation, the skeletons were stored in the Museo del Templo Mayor, Mexico City. After the removal of dirt with a brush and the cleaning of each bone sample with cotton-tipped applicators soaked in 5% ethanol, anthropometric measurements were taken from the skeletal remains by a team of two females under the direction of one of us (J.A.R., a male); these procedures were carried out wearing clean disposable latex gloves in the Anthropology Laboratory at the Museo del Templo Mayor. After the morphological studies, the bones were put into new polyethylene bags that were labeled and stored in cardboard boxes.

The reliability of the molecular sexing method was tested on two groups of skeletons whose sex could be reliably determined using morphometric data. The first group consisted of the nearly complete remains of an adult and those of the two adolescents aged 15 years from Tlatelolco (E31, E7, and E20, respectively, in table 1) and those of three pre-Columbian adults from other archaeological sites stored in the Museo del Templo Mayor, Mexico City. The pelvic bones of the two adolescents already showed a marked degree of sexual dimorphism. A previous osteological study of a pre-Hispanic population from Tenochtitlan demonstrated that the skeletons of adolescent individuals of comparable age to Tlatelolco's usually show an advanced degree of sexual differentiation, so their sex can be easily identified by standard morphometric analysis (Salas Cuesta 1982). The second group consisted of six adult skeletons that were excavated from a colonial chapel (sixteenth and seventeenth centuries) in Xcaret, Quintana Roo, in 1992 and stored in the Escuela Nacional de Antropología e Historia (ENAH), Mexico City.

Morphometric Analysis

The ages of 31 subadults and 1 adult were estimated using standard morphometric analyses including an evaluation of tooth calcification and eruption following the scheme proposed by Ubelaker (1989). Sex identification by standard morphometric analysis (Genovés 1959; Krogman and Iscan 1986; Ubelaker 1989) was carried out on the nearly complete skeleton of the adult (E31 in table 1) and the two adolescents (E7 and E20 in table 1) from Tlatelolco, the three adult skeletons stored in the Museo del Templo Mayor, and the six adult skeletons from Quintana Roo stored in the Escuela Nacional de Antropología e Historia.

DNA Extraction and Purification

The procedures for cleaning the bone samples and the precautions taken to avoid contamination with contemporary human DNA in the laboratory have been described elsewhere (González-Oliver et al. 2001). All the molecular work at the Universidad Nacional Autónoma de México was conducted by two females (I.D.C. and A.G.O.). Ribs were the source of

Table 1. Ancient DNA Extraction Methods Used at the Universidad Nacional Autónoma de México and Number of Successful Repeats Made for Each Sample

Sample ID	Age at Death[a]	Bone A	Bone B	Urn[b]	Replication of Results[c]
19–38	6 m ± 3	M1XY	K2Y		Yes
5–11	9 m ± 3	M1XY	K2Y	+	Yes
3–21	1 ± 4	K1Y	C1Y, K1X	+	Yes
14–27	1 ± 4	K2X	K2Y		Yes
20–33	1[d]	M1XY	K1Y		Yes
7–2	1.5 ± 6	K2Y	K1Y	+	Yes
4–1	2 ± 8	K1Y	K1Y		Yes
5–22	2 ± 8	M1XY	K1Y	+	Yes
20–32	2 ± 8	M1XY	K1Y		Yes
22–34	2 ± 8	M1XY	K1Y		Yes
23–36	2 ± 8	K1Y	K1Y	+	Yes
5–12	2[d]	M1Y	K1Y		Yes
19–19	4 ± 12	M1XY	M1Y		Yes
E30	4 ± 12	K2Y			Yes
3–15	4[d]	K1Y	K1Y		Yes
2–16	6 ± 24	K1XY	K1Y		Yes
E5	6 ± 24	K1Y	K1Y		Yes
8–3	8 ± 30	K1X	K1Y, K1Y	+	Yes
9–4	11 ± 30	M1XY	K1Y	+	Yes
E9–2	12 ± 30	K3Y	K1Y, K1Y		Yes
E7[e]	15 ± 36	K1Y, M1Y	K1Y		Yes
E20[e]	15 ± 36	K1Y	K1Y		Yes
11–13	Adult	K1Y	K1Y		Yes
11–18	Adult	C1Y	K1Y	+	Yes
E6	Adult	K1Y	K1Y		Yes
E-31[e]	Adult	C1Y, K1Y	NA		Yes
20–39	0 ± 2	M1XY		+	No
3–10	0[d]	K1Y		+	No
2–24	6 m ± 3	M1Y		+	No
14–40	6 m ± 3	K1X, K1XY		+	No
2–23	1[d]	M1Y	K2X	+	No
10–26	2 ± 8	M1XY		+	No
E37	2 ± 8	K1Y			No
2–25	3 ± 12	M1Y	K1X	+	No
E9–1	3 ± 12	K1Y			No
8–28	6[d]	M1XY		+	No
12–14	Adult	K1Y			No
E9-3	Adult	K1Y	NA		No

Note: Each aDNA extract is indicated by a capital letter, followed by the number of independent successful PCR amplifications and the specific amplicons obtained (X and/or Y). C, Chelex; K, Krings et al. (1997); M, Muñoz (González-Oliver et al. 2001); NA, not available. Individuals are ordered by age.

[a]Age at death in years (unless stated "m" for months) ± months.

[b]Individuals buried inside urns indicated with a plus sign (Guilliem 1999).

[c]Amplification of Y-specific product was replicated at least once.

[d]Taken from the original archaeological report (Guilliem 1999).

[e]Identified as male by morphometric analysis.

aDNA extracted from most samples in this study. The complete bone was used in most cases, but in a few cases in which the end of the rib had been broken, the rib was cut approximately 1 cm from the break, and the piece containing the break was discarded. In several cases, DNA was extracted from the neural arch of a vertebra.

Ancient DNA was extracted from most samples following either a previously described technique that has been extensively tested with mitochondrial DNA primers (González-Oliver et al. 2001) or the method described by Krings et al. (1997). A Chelex-100 resin extraction method (Faerman et al. 1995; Walsh, Metzger, and Higuchi 1991) was initially used in the analysis of a few samples because it is very simple, but it was subsequently abandoned because it provided too low a yield of DNA. Negative extraction controls, which contained all reagents except bone, accompanied each set of extractions and were analyzed in parallel with the samples. The extraction procedures were carried out in a dedicated HEPA-filtered laminar-flow cabinet in a different laboratory from that in which PCR amplifications were set up; the cabinet was cleaned

with 20% v/v Clorox bleach and UV-irradiated between experiments.

PCR Amplification

Amplification reactions were prepared in a dedicated HEPA-filtered laminar-flow cabinet within an isolated room in the pre-PCR laboratory, which was separate from the post-PCR laboratory. The amplification method described by Faerman et al. (1995) was modified by using the following primers: common primer (COM): 5′GCTCATATTATACTTGACAA-AGCA 3′; X-specific primer (Xsp): 5′GACGTCGGGTTTGAG-GTTCTC 3′; Y-specific primer (Ysp): 5′AGGTAAAATTACT-AACTTTGGGCA 3′. These primers were designed from the published amelogenin sequences (Nakahori, Takenaka, and Nakagome 1991) and produced smaller amplification products than the primers used by Faerman et al. (1995): 192 base pairs (bp) for the X-specific fragment (np 731–922) and 158 bp for the Y-specific fragment (np 587–744; fig. 2). Reactions were carried out in 25 μL containing 100 μM of each dNTP, 0.2 μM COM primer, 0.2 μM of either Ysp or Xsp primers (or 0.1 μM each of Ysp and Xsp primers, in multiplex reactions), 0.1 mg/mL bovine serum albumin (BSA), 2.5 mM $MgCl_2$, 1.5 units of AmpliTaq DNA polymerase Stoffel fragment or 1 unit of AmpliTaq Gold DNA polymerase, 2.5 μL of the appropriate 10 × buffer supplied by the manufacturer (PE Applied Biosystems), and 1–10 μL of DNA template. Forty cycles of amplification were carried out in a Perkin Elmer 480 cycler, each cycle consisting of 94°C for 30 s, 57°C for 30 s and 72°C for 1 min. Negative extraction and PCR controls were included in every set of PCR reactions to monitor possible contamination. The PCR products were electrophoresed on 14% nondenaturing polyacrylamide gels. The sizes of the PCR products were determined by comparison with known standards after staining with ethidium bromide.

Sequencing

The amplification products of selected samples were sequenced to confirm the results based on fragment analysis. These products were purified with the Wizard PCR Preps DNA Purification System (Promega) according to the manufacturer's recommendations. Five μL of the purified PCR product were sequenced in an automated sequencer using ABI Prism 310 dye terminator sequencing kits.

Test of Reliability of Molecular Results

After the PCR conditions were optimized using DNA of contemporary human males and females, the molecular sexing technique described above was conducted on DNA extracts from the two sets of skeletons whose sex had been identified by morphometric analysis. The samples were labeled with an arbitrary identification code and analyzed without knowledge of their sex.

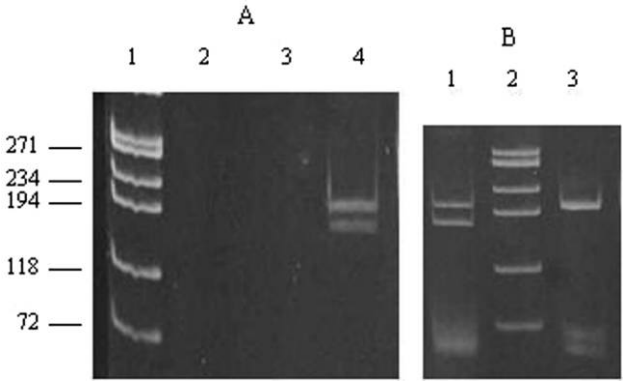

Figure 2. *A*, amplification products from an aDNA extract. Lanes A2 to A4, 1, 3, and 10 μL of DNA from sample E7, respectively. *B*, amplification products from contemporary controls. Lane B1, male human DNA; lane B3, female human DNA. Lanes A1 and B2, size standard, ϕX174 DNA/HaeIII fragments; sizes at left are in bp.

Confirmation of Sex Identification at UCD

Fifteen subadults whose amelogenin locus had been previously analyzed at the UNAM were reanalyzed at the Molecular Anthropology Laboratory at UCD. New bones were selected from the appropriate remains stored in the Museo del Templo Mayor, INAH, Mexico City, and sent to UCD.

DNA Extraction

Between 0.19 and 0.49 g of bone was removed from each bone (14 ribs and 1 vertebra). The samples were submerged in 6% sodium hypochlorite (full-strength commercial Clorox bleach) for 15 min (Kemp and Smith 2005). The bleach was removed by rinsing the sample with DNA-free water (Gibco). An extraction control, to which no bone was added, accompanied each set of extractions and was subjected to all of the steps that follow. The samples were then submerged in 2 mL of molecular-grade (DNA-free) 0.5 M EDTA, pH 8.0 (Gibco), for at least 48 h. Three mg of proteinase K were added to the samples, which were then incubated at 65°C for 4 h.

DNA was extracted from the proteinase K-digested samples using a three-step phenol/chloroform method: two extractions adding an equal volume of phenol : chloroform : isoamyl alcohol (25 : 24 : 1) to the EDTA followed by one extraction with an equal volume of chloroform : isoamyl alcohol (24 : 1). To aid removal of coextracted PCR inhibitors, DNA was precipitated from solution with isopropanol (Hänni et al. 1995) by adding one half volume of room-temperature 5 M ammonium acetate and, to the combined volume, one volume of room-temperature absolute isopropanol. The samples were then stored overnight for 8–14 h at room temperature.

The tubes were centrifuged for 30 min at 2,700 rpm to pellet the DNA. The liquid was discarded and the DNA pellet

was air dried for 15 min. The pellet was washed with 1 mL of 80% ethanol by vortexing for 30 sec, and then the tube was centrifuged for 30 min at 2,700 to repellet the DNA. The ethanol was poured off and the DNA pellet air dried for 15 min. To remove further coextracted PCR inhibitors, the DNA pellet was resuspended in 300 μL of DNA-free ddH_2O and extracted using the Wizard PCR Preps DNA Purification System (Promega) following the manufacturer's instructions (except that the DNA was finally eluted with 100 μL of ddH_2O). The final solution was stored at -20°C.

PCR Amplification and Analysis of Mitochondrial DNA from the Tlatelolco Remains

Before the sex of the individuals was determined, DNA isolated from the samples was screened for the polymorphisms that define the New World mitochondrial haplogroups A, B, C, and D (Forster et al. 1996; Schurr et al. 1990). The mitochondrial DNA (mtDNA) of the researcher (B.M.K.) who performed this work belongs to haplogroup U, which lacks these polymorphisms. The mtDNA of the only male who participated in the cleaning of the skeletons and the morphometric studies (J.A.R.) also lacks these polymorphisms.

Fifteen μL PCR amplification reactions contained 0.32 mM dNTPs, 1X PCR Buffer, 1.5 mM $MgCl_2$, 2.4 mM primers, 0.3 U of Platinum *Taq* (Invitrogen), and 1.5 μL of DNA template. Negative controls accompanied every set of PCR reactions to monitor the presence of contaminating DNA. Coordinates for primers have been previously described (Kaestle and Smith 2001). The PCR conditions were as follows: denaturation at 94°C for 3 min, then 40 consecutive cycles of 15-sec holds at 94°C, 55°C, and 72°C, and a final 3-min extension period at 72°C. Five μL of amplicons were separated on 6% polyacrylamide gels, which were stained with ethidium bromide and visualized under UV light to either confirm the successful amplification before restriction enzyme analysis or score the presence or absence of the 9-bp deletion. Restriction enzyme digestion of the remaining volume of amplicons was performed using 2 units of the appropriate enzyme (HaeIII for haplogroup A and AluI for both haplogroups C and D). These products were subjected to gel electrophoresis and visually inspected to assess digestion and assign haplogroups.

PCR Amplification and Analysis of the Sex Chromosomes from the Tlatelolco Remains

The Sullivan protocol was used to determine the sex of the individuals. The composition of the PCR reactions was identical to that listed above for determining mitochondrial haplogroups except that the primers used were Amel-A and Amel-B (Sullivan et al. 1993). PCR conditions were as follows: 94°C for 3 min, 60 cycles of 15-sec holds at 94°C, 55°C, and 72°C, and a final 3-min extension at 72°C. The amplicons were visualized and scored as described above, replicating the analysis of each extraction at least four times.

All of the samples were also screened at least once for two single nucleotide polymorphisms (SNPs) of the Y chromosome that define the Y-chromosome haplogroups Q-M242* and Q-M3*, respectively. The researcher who performed this work (B.M.K.) lacks both of these SNPs. PCR reactions contained 1.5–3.0 μL of DNA template, 2.4 μL of 2 mM dNTPs, 1.5 μL of 10X PCR Buffer, 0.45 μL $MgCl_2$ (50mM), 1.8 μL of each primer (20 μM), 0.06 μL of Platinum *Taq* (Invitrogen), and sufficient DNA-free ddH_2O (Gibco) to adjust the total reaction volume to 15 μL. Primers used to amplify the M-242 region were M-242-Forward (5′AACTCTTGATAAACCGTGCTG3″) and M-242-Reverse (5′AACACGTTAAGACCAATGCTAA). Primers used to amplify the M-3 region were M-3-Forward (5′-TAATCAGTCTCCTCCCAGCA-3′) and M-3-Reverse (5′AGGTACCAGCTCTTCCCAATT). In some PCR reactions, M-3-Forward (Short) (5′CGCGGGATAAATGTGGCCAAGTTTT3′) was substituted for M-3-Forward to create 105-bp amplicons (instead of 200-bp amplicons when amplified with M-3-Forward). The PCR conditions for M-242 were as follows: 94°C for 3 min, 60 cycles of 15-sec holds at 94°C, 56°C, and 72°C, and a final 3-min extension at 74°C. The PCR conditions for M-3 were the same as those used for M-242 except that an annealing temperature of 59°C was employed for amplifying M-3. When M-3-Forward (Short) was used, the annealing temperature was 50°C. Confirmation of PCR amplification and restriction enzyme digestion was performed as described above.

Results

UNAM Results

The ages of 31 subadults and 1 adult were estimated by standard morphometric analysis of the skeletal remains (table 1). Most of the subadults (66%) were children up to three years old. The ages of 6 subadults could not be assessed because of the absence of teeth; for these individuals, the age recorded in table 1 is a gross estimation taken from the original archaeological report (Guilliem 1999). The size of the chest bones of 5 fragmentary skeletons suggested that they were adults, but their ages could not be estimated. The sex of 1 adult and the 2 adolescents aged 15 could be identified by morphometric analysis as males; in all three cases, the molecular results confirmed this assignment (table 1).

DNA from four of six individuals used in the first test of reliability of the molecular technique could be successfully amplified and in each case corroborated the morphometric results: all four individuals were males (data not shown). DNA of three of the six individuals from the second group could be successfully amplified and, again, in each case confirmed the morphometric results: two were females and one was a male (data not shown).

Amplifiable DNA could be obtained from 32 of the 37 subadults and all 6 adults from Tlatelolco. The Chelex-100 extraction method provided a low success rate, but the results obtained were confirmed using the other two extraction meth-

ods employed (González-Oliver et al. 2001; Krings et al. 1997). Although both extraction methods showed good success rates (table 1), we preferred to use the Krings method to avoid the electrophoresis step required by the other method (González-Oliver et al. 2001). In spite of the greater efficiency of these two methods compared with the Chelex-100 method, no DNA could be amplified from more than 60% of all aDNA extracts.

The 32 subadults and 6 adults from whom DNA could be recovered were initially identified as males by the presence of the Y-chromosome amplicon. However, these results could be replicated in only 26 individuals (60%; table 1); 22 of the 26 were replicated in separate extracts from different bones, 2 in separate extracts from the same bone, and 2 in separate PCRs from the same extract. Direct sequencing of selected samples confirmed the results based on scoring amplicons by gel electrophoresis. DNA extraction controls and PCR controls were consistently negative.

UCD Results

Mitochondrial DNA was successfully amplified from 14 of the 15 individuals (93.3%) analyzed at UCD for confirmation of the sex identification made at the UNAM. The mitochondrial DNA of 8 of these individuals (57.1%) belong to haplogroup A, 3 (21.4%) to haplogroup B, 1 (7.1%) to haplogroup C, and 2 to haplogroup D (14.3%). These haplogroup frequencies are statistically indistinguishable from those reported in a previous sample of 23 Aztec remains from Tlatelolco (χ^2 test of homogeneity, $\chi^2 = 0.646$, $p = 0.8858$; Kemp et al. 2005). Molecular sex identification was possible for 9 of the 15 (60%) individuals. Of these 9 individuals, 1 was determined to be a female (11.1%) and 8 were determined to be males (88.9%). It was possible to screen 6 of the 15 samples for the M3 and M242 Y-chromosome SNPs. Four of these 6 individuals exhibited both the M3 and M242 polymorphisms and, therefore, belong to Y-chromosome haplogroup Q-M3*, which is restricted to the New World and exhibits particularly high frequencies in contemporary Mesoamerican populations (Malhi et al. in review). The fifth individual (sample 7-2) lacked the M3 SNP and therefore does not belong to haplogroup Q-M3*. However, it could not be screened for the M242 marker and therefore could belong to haplogroup Q-242*. The last individual (sample 14-27) exhibited the M242 marker but could not be screened for the M3 marker. Therefore, it could still belong to haplogroup Q-M3*.

Discussion

The identification of an individual as male was based on the presence of the Y-chromosome-specific amplification product. To reduce false sex identifications, we carried out several replicate PCRs with the same ancient DNA extracts and replicated the results from independent extracts on separate bones belonging to a single individual. Fifteen of these were carried out in a separate laboratory at UCD using completely different methods. In only one case did the sex determination of an individual (8–28) provide contradictory results, probably the result of allelic dropout. Although we did not quantitatively evaluate differential amplification efficiency of the X- and Y-specific products at the UNAM, we frequently observed allelic dropout in multiplex PCRs of aDNA—that is, one of the expected amplification products, either the Y-specific product or the X-specific product, was absent. In contrast, male contemporary DNA controls always showed the presence of both amplicons in multiplex PCRs. Although the Sullivan amelogenin test (Sullivan et al. 1993) works well with modern and forensic DNA samples, some researchers have reported problems using these primers to amplify aDNA because of allelic dropout (Brown 2001; Lassen, Hummel, and Hermann 1996). In fact, in the studies performed at UCD, 13 of 22 (59%) amelogenin amplifications (table 2) of samples believed to be male demonstrated allelic dropout of the X- or Y-chromosome amplicon. Thus, we cannot rule out the occurrence of Y-chromosome dropout in the sample thought to be female. The sex identification of the samples analyzed at UCD is supported by the presence of the M3 and M242 SNPs in over half the individuals determined to be male on the basis of screening of the amelogenin gene alone. It should be noted that the sample (20–32) that did not amplify for mtDNA at UCD also failed to amplify for any sex chromosome marker, an expected result given the high copy number of the mtDNA genome in comparison with the nuclear genome. Furthermore, the frequencies of mtDNA haplogroups isolated from these remains are statistically indistinguishable from previously sampled Aztecs from the same site (Kemp et al. 2005). Thus, the weight of the evidence suggests that the DNA from these 15 remains was of pre-Columbian origin and does not represent modern contaminating DNA.

The amplification of the Y-specific product could be replicated in 26 of 43 (60%) individuals analyzed at the UNAM. For the remaining 12 individuals for whom DNA could be recovered for analysis, the results could not be replicated with the available samples (table 1). The 60% success rate of sex identification obtained in this study is moderately high and probably resulted from the favorable state of preservation of the skeletal remains from this archaeological site and the use of multiple methods of aDNA extraction.

In the Aztec tradition, Tlaloc, the principal rain god, was helped by a myriad of gods with small bodies, collectively called the Tlaloque. These little gods were thought to live in the hills and mountains and control the rain (Román 1990). Ehecatl-Quetzalcoatl, the Aztec god of wind, was considered to be one of the Tlaloque, and he served as the forerunner who blew obstacles from the road to make way for the rain. The cult dedicated to Ehecatl-Quetzalcoatl as a rain god and

Table 2. Results of Studies Performed at the University of California, Davis

Sample ID	mtDNA Haplogroup	Amelogenin 1	Amelogenin 2	Amelogenin 3	Amelogenin 4	M3	M242	Y-Chromosome Haplogroup	Sex
19–38	A				112			Unknown	M
5–11	D	112	106	106/112	112			Unknown	M
14–27	B			112	106		+	Q-M242*?	M
20–33	A					+	+	Q-M3*	M
7–2	D			112	112	–		Unknown	M
4–1	A							Unknown	Unknown
5–22	C		106	106/112	106/112	+	+	Q-M3*	M
20–32	Unknown							Unknown	Unknown
22–34	A							Unknown	Unknown
E37	A							Unknown	Unknown
3–15	A	106		106/112	106/112	+	+	Q-M3*	M
2–16	A		106/112					Unknown	M
E5	B	106	106/112	112	112	+	+	Q-M3*	M
8–28	A		106	106				Unknown	F
E7	B							Unknown	Unknown

Note: Unknown mtDNA haplogroup corresponds to failure of DNA to be extracted from the sample. Amelogenin 1–4 represents four attempts to amplify the amelogenin alleles. The sizes 106 and 112 represent the X- and Y-chromosome specific amplicons. A plus sign under M3 and M242 represents the presence of the defining polymorphisms of haplogroups Q-M3* and Q-M242*, respectively. A minus sign represents the lack of the defining polymorphism. Blank fields under Amelogenin 1–4, M3, and M242 represent missing data due to the failure of PCR to amplify DNA. The Y-chromosome haplogroup and sex of the individual, when known, are listed. Individuals are ordered by age.

the exceptional characteristics of the massive offering found in his temple at Tlatelolco prompted Guilliem (1999) to hypothesize that this ceremonial complex was the site of an extraordinary ceremony carried out during the great drought and famine of AD 1454–57, a date consistent with the founding of this temple. Considering that the Tlaloque were also patrons of several diseases, it has been proposed that the Aztecs believed that the gods themselves selected these children for sacrifice, the selection being manifested by the disease symptoms exhibited by the children. Osteopathological (Román 1991) and dental pathological (Román and Rodríguez 1997) analysis of children sacrificed at Tenochtitlan and Tlatelolco showed that many were children whose health conditions were precarious in varying degrees; however, no quantitative comparison between the prevalence of the pathologies found in the remains of these children and that found in the general population was offered.

According to Broda (1971), the victims chosen for sacrifice in ancient Aztec rituals became the living impersonation of the god to whom they were offered. In the case of the Tlaloque, children were frequently selected to personify these little deities. It has also been proposed that children were chosen as sacrificial victims because their youth provided them the purity to communicate propitiously with the gods and obtain their favor (López Austin 1984, vol. 1:324). Our results lend support to Broda's proposal because, since Ehecatl-Quetzalcoatl was a male deity, male victims would better personify him than female victims. Thus, the results of the morphological and molecular studies reported here provide support for the archaeological interpretation of the ceremony conducted at the temple dedicated to Ehecatl-Quetzalcoatl at Tlatelolco.

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References Cited

Bass, W. M. 1989. *A laboratory and field manual of the human skeleton.* St. Louis: Missouri Archaeological Society.

Benavente, T. 1979. *Historia de los Indios de la Nueva España.* Mexico City: Editorial Porrúa.

Broda, J. 1971. Las fiestas Aztecas de los dioses de la lluvia. *Revista Española de Antropología* 6:245–327.

Brown, K. A. 1998. Gender and sex: What can ancient DNA tell us? *Ancient Biomolecules* 2:3–15.

———. 2001. Identifying the sex of human remains by ancient DNA analysis. *Ancient Biomolecules* 3:215–25.

Durán, D. 1984. *Historia de las Indias de Nueva España e Islas de la Tierra Firme.* Mexico City: Editorial Porrúa.

Faerman, M., D. Filon, G. Kahila, C. L. Greenblatt, P. Smith, and A. Oppenheim. 1995. Sex identification of archaeological human remains based on amplification of the X and Y amelogenin alleles. *Gene* 167:327–32.

Forster, P., R. Harding, A. Torroni, and H. J. Bandelt. 1996. Origin and evolution of native American mtDNA variation:

A reappraisal. *American Journal of Human Genetics* 59: 935–45.

Genovés, T. S. 1959. *Diferencias sexuales en el hueso coxal.* Mexico City: Universidad Nacional Autónoma de México.

González-Oliver, A., L. Márquez-Morfín, J. C. Jiménez, and A. Torre-Blanco. 2001. Founding Amerindian mitochondrial DNA lineages in ancient Maya from Xcaret, Quintana Roo. *American Journal of Physical Anthropology* 116:230–35.

Götherstrom, A., K. Liden, T. Ahlström, M. Köllersjö, and T. A. Brown. 1997. Osteology, DNA, and sex identification: Morphological and molecular sex identification of five Neolithic individuals from Ajvide, Gotland. *International Journal of Osteoarchaeology* 7:71–81.

Guilliem, S. 1999. *Ofrendas a Ehécatl-Quetzalcoatl en México-Tlatelolco: Proyecto Tlatelolco 1987–1996.* Mexico City: Instituto Nacional de Antropología e Historia.

Hänni, C., T. Brousseau, V. Laudet, and D. Stehelin. 1995. Isopropanol precipitation removes PCR inhibitors from ancient bone extracts. *Nucleic Acids Research* 23:881–82.

Kaestle, F. A., and D. G. Smith, 2001. Ancient mitochondrial DNA evidence for prehistoric population movement: The Numic expansion. *American Journal of Physical Anthropology* 115:1–12.

Kemp, B. M., A. Resendez, A. R. Roman Berrelleza, R. S. Malhi, and D. G. Smith. 2005. An analysis of ancient Aztec mtDNA from Tlatelolco: Pre-Columbian relations and the spread of Uto-Aztecan. In *Biomolecular archaeology: Genetic approaches to the past,* ed. D. M. Reed. Carbondale: Southern Illinois University Press.

Kemp, B. M., and D. G. Smith. 2005. Use of bleach to eliminate contaminating DNA from the surfaces of bones and teeth. *Forensic Science International* 154:53–61.

Krings, M., A. Stone, R. W. Schmitz, H. Krainitzki, M. Stoneking, and S. Pääbo. 1997. Neandertal DNA sequences and the origin of modern humans. *Cell* 90:19–30.

Krogman, W. M., and M. Y. Iscan. 1986. *The human skeleton in forensic medicine.* 2d edition. Springfield, Ill.: Charles C. Thomas.

Lassen, C., S. Hummel, and B. Hermann. 1996. PCR based sex identification of ancient human bones by amplification of X- and Y-chromosomal sequences: A comparison. *Ancient Biomolecules* 1:25–33.

López Austin, A. 1984. *Cuerpo humano e ideología.* Vol. 1. Mexico City: Universidad Nacional Autónoma de México.

Meyer, E., M. Wiese, H. Bruchhaus, M. Claussen, and B. Klein. 2000. Extraction and amplification of authentic DNA from ancient human remains. *Forensic Science International* 113: 87–90.

Nakahori, Y., O. Takenaka, and Y. Nakagome. 1991. A human X-Y homologous region encodes "amelogenin." *Genomics* 9:264–69.

O'Rourke, D. H. 2007. Ancient DNA and its application to the reconstruction of human evolution and history. In *Anthropological genetics: Theory, methods, and applications*, ed. M. Crawford. Cambridge: Cambridge University Press.

Pääbo, S. 1989. Ancient DNA: Extraction, characterization, molecular cloning, and enzymatic amplification. *Proceedings of the National Academy of Sciences U.S.A.* 86:1939–43.

Román, J. A. 1990. *Sacrificio de niños en el Templo Mayor.* Mexico City: Instituto Nacional de Antropología e Historia.

———. 1991. A study of skeletal material from Tlatelolco. In *To change place: Aztec ceremonial landscapes*, ed. D. Carrasco, 9–19. Niwot: University Press of Colorado.

Román, J. A., and M. C. Rodríguez. 1997. Las patologías dentales en individuos localizados en ofrendas a los dioses de la lluvia. In *El cuerpo humano y su tratamiento mortuorio*, ed. E. Malvido, G. Pereira, and V. Tiesler, 213–40. Mexico City: Instituto Nacional de Antropología e Historia-CEMCA.

Sahagún, B. 1975. *Historia general de las cosas de Nueva España.* Mexico City: Editorial Porrúa.

Salas Cuesta, M. E. 1982. *La población de México-Tenochtitlan: Estudio de osteología antropológica.* Colección Científica 126. Mexico City: Instituto Nacional de Antropología e Historia.

Schmidt, D., S. Hummel, and B. Herrman. 2003. Brief communication: Multiple X/Y-PCR improves sex identification in aDNA analysis. *American Journal of Physical Anthropology* 121:337–41.

Schurr, T. G., S. W. Ballinger, Y. Y. Gan, J. A. Hodge, D. A. Merriwether, D. N. Lawrence, W. C. Knowler, K. M. Weiss, and D. C. Wallace. 1990. Amerindian mitochondrial DNAs have rare Asian mutations at high frequencies, suggesting they derived from four primary maternal lineages. *American Journal of Human Genetics* 46:613–23.

Stone, A. C., G. R. Milner, S. Pääbo, and M. Stoneking. 1996. Sex determination of ancient human skeletons using DNA. *American Journal of Physical Anthropology* 99:231–38.

Sullivan, K. M., A. Mannucci, C. P. Kimpton, and P. Gill. 1993. A rapid and quantitative DNA sex test: Fluorescence-based PCR analysis of X-Y homologous gene amelogenin. *BioTechniques* 15:636–41.

Ubelaker, D. H. 1974. *Reconstruction of demographic profiles from ossuary skeletal samples.* Washington, D.C.: Smithsonian Institution.

———. 1989. *Human skeletal remains.* Washington, D.C.: Taraxacum Press.

Walsh, P. S., D. A. Metzger, and R. Higuchi. 1991. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *BioTechniques* 10:506–13.