

The Genetic Impact of Aztec Imperialism: Ancient Mitochondrial DNA Evidence From Xaltocan, Mexico

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ABSTRACT In AD 1428, the city-states of Tenochtitlan, Texcoco, and Tlacopan formed the Triple Alliance, laying the foundations of the Aztec empire. Although it is well documented that the Aztecs annexed numerous polities in the Basin of Mexico over the following years, the demographic consequences of this expansion remain unclear. At the city-state capital of Xaltocan, 16th century documents suggest that the site's conquest and subsequent incorporation into the Aztec empire led to a replacement of the original Otomí population, whereas archaeological evidence suggests that some of the original population may have remained at the town under Aztec rule. To help address questions about Xaltocan's demographic history during this period, we analyzed ancient DNA from 25 individuals recovered from three houses rebuilt over time and occupied between AD 1240

and 1521. These individuals were divided into two temporal groups that predate and postdate the site's conquest. We determined the mitochondrial DNA haplogroup of each individual and identified haplotypes based on 372 base pair sequences of first hypervariable region. Our results indicate that the residents of these houses before and after the Aztec conquest have distinct haplotypes that are not closely related, and the mitochondrial compositions of the temporal groups are statistically different. Altogether, these results suggest that the matriline present in the households were replaced following the Aztec conquest. This study therefore indicates that the Aztec expansion may have been associated with significant demographic and genetic changes within Xaltocan. *Am J Phys Anthropol* 000:000–000, 2012. © 2012 Wiley Periodicals, Inc.

The collapse of the Toltec state in the 12th century prompted the emergence of numerous city-states in the Basin of Mexico. These city-states fought one another until power was consolidated into a few larger polities, and by the early 15th century, Azcapotzalco (capital of the Tepanec state) took control of most of the Basin of Mexico. However, in 1428 the city-states of Tenochtitlan, Texcoco, and Tlacopan forged the Triple Alliance, reducing the influence of the Tepanec state and laying the foundations of the Aztec empire, which conquered a large number of polities in Mesoamerica over the following years (Brumfiel, 1983; Berdan and Smith, 2003a,b; Smith and Berdan, 2003).

The spread of the Tepanec state and the later expansion of the Aztec empire have been the subject of much research, but their demographic consequences remain unclear. In this study, we examined ancient DNA (aDNA) from human remains recovered at Xaltocan, a town located 35 km north of Mexico City (Fig. 1) that was conquered by the Tepanecs and later integrated into the Aztec empire. We used aDNA data from three Xaltocan households in conjunction with historical and archaeological evidence to investigate whether any demographic and genetic changes occurred in association with these events.

Although historical documents state that Xaltocan was founded around AD 1100, when a group of Otomí-speaking people migrated into the northern Basin of Mexico from the west (Gibson, 1964), archaeological evidence places the foundation of the town earlier, around AD 900

(Brumfiel, 2005a). At that time, Xaltocan was a relatively small island (~800 × 400 m) rising 5–6 m above the bed of the now drained Lake Xaltocan (Brumfiel, 2005d; Morehart and Eisenberg, 2010).

In the 11th–14th centuries, the town grew into an important city-state that collected tribute from neighboring communities (Carrasco-Pizana, 1987; Brumfiel, 2005a,c) and served as the capital of the Otomí state (Gibson, 1964; Brumfiel, 2005d; De Lucia, 2010). Xaltocan's preeminence in the Basin of Mexico has been attributed to the availability of water, the existence of abundant lake resources (i.e., fauna, plants, and salt), rich soil, productive agricultural practices, and a fruitful market network with nearby cities (Brumfiel, 2005c; Hodge and Neff, 2005; Morehart and Eisenberg, 2010). At its peak,

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Fig. 1. Map of the Basin of Mexico in pre-Hispanic times. The dotted areas represent lakes [from Rodríguez-Alegría (2010)].

Xaltocan may have had a population of nearly 5,000 individuals (Sanders et al., 1979).

By the mid-13th century, Xaltocan became involved in a war against the neighboring Tepanec kingdom of Cuauhtitlan (Código Chimalpopoca, 1975; Carrasco-Pizana, 1987). This conflict resulted in a lengthy struggle that gradually reduced Xaltocan's dominions (Código Chimalpopoca, 1975). According to 16th century documents, Cuauhtitlan finally vanquished Xaltocan in 1395 with the aid of other Tepanec allies (Gibson, 1964; Código Chimalpopoca, 1975; Carrasco-Pizana, 1987). The Código Chimalpopoca (1975) also asserts that the original Otomí population fled Xaltocan at this time, leaving the town unpopulated. A native history recorded by Alva Cortés Ixtlilxóchitl (1975–1977, II:36) in the early 17th century includes an eyewitness account in which commoners were seen fleeing the site escorted by Xaltocan soldiers, thereby supporting the abandonment account.

The emerging Aztec empire annexed Xaltocan in 1428, and Tenochtitlan (the capital of the empire) assigned rulers to govern the town (Carrasco-Pizana, 1987; Brumfiel, 2005d). According to the *Anales de Cuauhtitlan* [(1939: Par. 628, 1017–1021), as cited in Hicks (1994: 67)], one of these rulers repopulated Xaltocan in 1435 with people who “were not the descendants of the original settlers, but came from other places.” The new population was multiethnic, including members of the Acolhua, Colhua, Tenochca, and Otomí ethnic groups, and came from a number of places in central Mexico, including Ixayoc-tonco, Totollan, Tlapallan, Tlilhuacan, and Ixayoc (*Anales de Cuauhtitlan*, 1939). Court testimony from a 1530s lawsuit (AGI Justicia 123/2, 1536) affirms the site's resettlement by the Aztec ruler [see also Hicks (1994)]. Hence, colonial documents clearly state that the

Tepanec and Aztec conquests led to a population replacement at Xaltocan.

However, archaeological evidence may contradict this account of the population history of Xaltocan after the town lost the war against Cuauhtitlan in 1395. Recent archaeological finds suggest a significant degree of population continuity after the defeat and even following incorporation into the Aztec empire (Overholtzer, 2012). For instance, radiocarbon analyses indicate that five burials likely date between 1395 and 1435, which calls into question the assertion that Xaltocan remained uninhabited during that period (Overholtzer, 2012). In addition, during the Aztec period, houses were built and burials were interred in the same locations as those used by the earlier Otomí residents (Overholtzer, 2011). Because new settlers arriving at a site 40 years after its abandonment may not share spatial norms of practice, this evidence suggests that at least some of the original commoner Otomí population may have remained at Xaltocan after the defeat in 1395. The historical account of a complete population replacement may therefore be inaccurate, perhaps because elites in the pre-Hispanic and early colonial periods overstated their role in the conquest and reformation of the site under empire (Overholtzer, 2012). The archaeological evidence does not preclude the possibility of a demographic change, however, because it is possible that elites and/or some of the commoner population fled. It is also possible that some newcomers were sent by the Aztec ruler to farm, fish, and produce salt and other goods, and these newcomers presumably would have intermarried with the existing population to form the taxpaying base for the empire.

Using aDNA to test hypotheses about demographic changes

Ancient DNA (aDNA) studies can be used to help investigate Xaltocan's demographic history and possible genetic changes in this town during the imperial Aztec transition. DNA sampled from burials that predate and postdate a hypothesized demographic transition can be used to evaluate if such an event occurred and, if so, the magnitude of change (Kaestle and Horsburgh, 2002; Ramakrishnan et al., 2005; Smith et al., 2009; Mourier et al., 2012). For example, temporal genetic sampling has helped elucidate past changes in the size, composition, and structure of other populations and has helped investigate hypothesized population replacements (Kaestle and Smith, 2001; Lewis et al., 2007; Cabana et al., 2008; Kemp et al., 2009; Smith et al., 2009; Snow et al., 2010; O'Fallon and Fehren-Schmitz, 2011).

aDNA studies have traditionally focused on mitochondrial DNA (mtDNA) because several copies of mtDNA are present in each mitochondrion, and, in turn, some cells may contain thousands of mitochondria (Jobling et al., 2004). Because aDNA studies rely on scarce and degraded genetic material, the fact that each cell contains numerous copies of mtDNA substantially increases the chances of amplifying the target sequence. The mitochondrial genome is maternally inherited and does not undergo recombination, thus constituting a useful tool for studying maternal ancestry (Eshleman et al., 2003). In addition, because mtDNA has a mutation rate about 10% higher than that of the nuclear genome and, within mtDNA, the 1,120 base pair (bp) control region has an even higher mutation rate (Eshleman et al., 2003; Jobling et al., 2004), the mtDNA control region is especially useful for genetic studies of population history that span relatively short periods of time.

Native Americans exhibit at least 15 founding mitochondrial lineages, of which the most common are haplogroups A2, B2, C1, D1, and X2 (Perego et al., 2010). These lineages have been traditionally distinguished by specific restriction fragment length polymorphisms (RFLP) or a 9-bp deletion in the mitochondrial genome, as well as by mutational motifs in the mtDNA control region (Schurr et al., 1990; Torroni et al., 1992, 1993; Brown et al., 1998; Smith et al., 1999). Likewise, additional mutations beyond the haplogroup-defining ones found within the control region differentiate sequences from the same haplogroup into different haplotypes. Haplogroup and haplotype data can serve as an important tool for studying genetic relationships among human populations, with similar haplogroup frequencies and/or shared haplotypes indicating recent common ancestry (Kaestle and Horsburgh, 2002; Bolnick and Smith, 2007). In Mesoamerica, most populations are characterized by high frequencies of haplogroup A2, lower frequencies of haplogroups B2, C1, and D1, and the absence of haplogroup X2 (Torroni et al., 1994; Peñalosa-Espinosa et al., 2007; Sandoval et al., 2009; Kemp et al., 2010). aDNA studies of Late Classic (AD 600–800) and Postclassic Maya (AD 900–1521) samples from Xcaret (González-Oliver et al., 2001) and Late Postclassic Aztec (AD 1325–1521) samples from Tlatelolco (Kemp et al., 2005) indicate the relative antiquity of this overall pattern in the region (Raff et al., 2011).

In this study, we used ancient mtDNA to investigate whether the Tepanec conquest of Xaltocan and its subsequent incorporation into the Aztec empire were associ-

ated with any demographic changes at the household level in Xaltocan. We report mtDNA data from human remains recovered as part of an archaeological investigation of three Xaltocan houses that were occupied and rebuilt over several centuries (AD 1240–1521; Overholtzer, 2012). The excellent archaeological context and radiometric data available for these samples facilitate a nuanced and fine-grained examination of the imperial transition and its demographic consequences at the scale of the household, which is “the level at which social groups articulate directly with economic and ecological processes,” and the locale in which imperialism was experienced (Wilk and Rathje, 1982: 618). Moreover, individuals and households collectively form society as a whole, and their practices and decisions are what drive broader political, economic, and demographic processes. We therefore divided the remains into two temporal groups that predate and postdate the Tepanec conquest of Xaltocan and investigated the patterns of genetic diversity within and between these groups. If there was genetic continuity across the imperial Aztec transition at Xaltocan, we would expect to see close genetic relationships between the preconquest and postconquest residents of these houses. On the other hand, if the imperial Aztec transition was associated with demographic and genetic changes at Xaltocan, then this event is predicted to coincide with a genetic shift in the sampled households, such that the preconquest residents of the houses were not closely related to the postconquest residents.

MATERIALS AND METHODS

Samples

We sampled skeletal remains (teeth or bone) from 26 individuals recovered during archaeological research in Xaltocan (Table 1). Excavations at this site, which covers about 68 hectares where the modern town of Xaltocan is located, began in 1987 (Brumfiel, 2005a,b,d). Of the 26 individuals, 21 were recovered from Operation Este, a group of excavation units located on the eastern edge of the site that was excavated by Lisa Overholtzer in 2009 and 2010. These individuals were recovered from two mounds containing stratified domestic deposits, and the archaeological evidence indicates that the dead were buried in the patios outside two neighboring houses. The other five individuals were recovered from a separate mound containing stratified domestic deposits that was excavated in Operations Y2 and Y3 in 2003 (Brumfiel, 2007). These burials are also thought to represent the interment of household members in the exterior patio of their house (Overholtzer, 2012). Archaeological and historical evidence suggests that all 26 individuals belonged to the nuclear or extended families that inhabited the three houses. The ages at death of the individuals analyzed in this study span the life course (from fetuses to elders), although it is unlikely that they represent all residents of the houses because fewer burials were recovered than would be expected given the length of occupation. It is likely that residents selected specific family members for interment in household space.

Of the 26 individuals, 11 were sampled for radiocarbon dating, and the radiocarbon dates were entered into a Bayesian statistical model of the occupation of the houses. The intercepts for the resulting dates range from about AD 1325–AD 1475 (Table 1). Approximate dates were determined for the remaining samples based on stratigraphy and the style of pottery placed in the

TABLE 1. *Xaltocan samples analyzed in this study*

Sample ID	Age at death ^a	Radiocarbon date (AD) ^b	Haplogroup	Haplotype ^c
<i>Preconquest</i>				
E14.3	Birth–3 months	1310–1350	D1	223T, 292T, 325C, 362C
E14.4	7 months ± 3 months	NA	D1	223T, 292T, 325C, 362C
E14.5	10 months–1 year	NA	D1	223T, 292T, 325C, 362C
E14.7	9 months–1 year	1300–1350	B2	182C, 183C, 189C, 217C, 295T
E25.2	9 months–1 year	1300–1360	A2	111T, 223T, 290T, 319A, 335G
Y2.3	NA	NA	A2	111T, 223T, 290T, 319A, 344T, 362C
Y2.7	NA	NA	B2	182C, 183C, 189C, 217C
Y3.6	NA	NA	B2	183C, 189C, 194C, 195C, 217C, 362C
Y3.8	NA	NA	A2	95T, 111T, 223T, 290T, 319A, 326G, 362C
Y3.10A	NA	NA	D1	223T, 292T, 325C, 362C
<i>Postconquest</i>				
E6.1	14 years ± 2 years	1410–1450	B2	182C, 183C, 189C, 217C, 357C
E7.1	5 years ± 1.5 years	1400–1450	A2	93C, 111T, 136C, 223T, 290T, 311C, 319A, 362C
E8.1	30–40 years	NA	A2	111T, 183C, 189C, 223T, 290T, 291T, 319A, 362C
E8.2	More than 50 years	NA	A2	93C, 111T, 136C, 223T, 290T, 311C, 319A, 362C
E8.3	30–40 years	NA	A2	93C, 111T, 136C, 223T, 290T, 311C, 319A, 362C
E8.4	1–3 months	1390–1440	A2	93C, 111T, 136C, 223T, 290T, 311C, 319A, 362C
E8.5	30–40 years	1390–1440	A2	93C, 111T, 136C, 223T, 290T, 311C, 319A, 362C
E10.1	20–35 years	NA	A2	172C, 223T, 290T, 319A, 362C
E10.2	More than 50 years	1400–1450	A2	93C, 111T, 136C, 223T, 290T, 311C, 319A, 362C
E14.1	8–9 years	NA	B2	183C, 189C, 217C, 258C, 260.1T ^d
E14.2	1 month	1430–1520	A2	111T, 192T, 223T, 290T, 319A, 362C
E14.6	30–35 years	1430–1500	B2	182C, 183C, 189C, 217C, 357C
E30.A	Fetus–birth	NA	D1	223T, 325C, 362C
E30.3	30–35 years	NA	C1	172C, 223T, 298C, 325C, 327T
E34.1	Adult	1330–1430	D1	223T, 325C, 362C

^a NA, not available.

^b NA, not available. When classifying the samples with radiocarbon dates as pre- or postconquest, their archaeological context was also taken into account.

^c Sequences from nps 16,011–16,382. Mutations are identified with reference to the Cambridge Reference Sequence (Anderson et al., 1981; Andrews et al., 1999).

^d Insertion between nucleotide positions 16,260 and 16,261.

graves. Burials associated with Aztec II Black-on-Orange ceramic deposits are thought to date between 1240 and 1350, whereas burials associated with Aztec III Black-on-Orange ceramic deposits are thought to date between 1350 and 1521. For the purpose of this study, we divided the sampled individuals into two temporal groups corresponding to the Aztec II period ($n = 10$) and the Aztec III period ($n = 16$). Because Aztec II pottery predates the conquest of Xaltocan, and Aztec III pottery roughly postdates the conquest of Xaltocan in 1395, we refer to these temporal groups as preconquest and postconquest groups. Although it is possible that some Aztec III burials may have occurred before 1395, the eight Aztec III burials that have been radiocarbon dated are statistically unlikely to date to the preconquest period. Seven of these eight burials have radiocarbon dates that fall squarely in the post-1395 range with 95% confidence. Although one Aztec III burial might have occurred earlier (E34.1), it has a 72% likelihood of dating after 1380. Thus, all Aztec III burials likely date to the postconquest period. It is important to note, however, that the exact date for the conquest of Xaltocan is not known with certainty. The date of 1395 was first written down in the 16th century (Código Chimalpopoca, 1975), so it should be treated as an approximation.

We also collected data for modern Mesoamerican populations from the published literature for comparison. According to ethnohistorical documents, the preconquest inhabitants of Xaltocan were Otomí, while the postconquest population was multiethnic but largely Nahua. Thus, we incorporated data on four modern Otomanguean-speaking populations (because the Otomí lan-

guage belongs to the Otomanguean language family) and six modern Uto-Aztecan populations (because the Nahuatl language belongs to the Uto-Aztecan language family; Table 2) into some analyses (see below).

Finally, we collected saliva or buccal swab samples from 11 archaeologists who participated in the excavations when the skeletal remains were recovered and from all researchers working in the aDNA laboratory at the University of Texas at Austin.

DNA extraction and purification

We extracted aDNA following Bolnick et al.'s (2012) nondestructive extraction protocol. To remove surface contamination, we submerged each sample in 6% sodium hypochlorite (full strength bleach) for 15 min (Kemp and Smith, 2005), rinsed it twice with DNA-free HPCL-grade water, irradiated it with 254-nm ultraviolet light (UV) for 10 min per side, and left it to dry at room temperature. We then transferred each sample to a 15-ml polypropylene tube and incubated it at room temperature in 10 ml of extraction solution (0.45 M EDTA and 0.25 mg ml⁻¹ proteinase K, pH 8.0) while rocking gently overnight. Following incubation, we poured the extraction solution into a separate tube without disturbing the sample and then rinsed the tooth or bone three times with DNA-free HPLC-grade water and air-dried it at room temperature. We purified the DNA left in the extraction solution using the silica/guanidium thiocyanate method published by Rohland and Hofreiter (2007) and then eluted it in 70 μ l of DNA-free HPLC-grade water.

TABLE 2. Haplogroup frequencies

Population	Language family	Haplogroup N ^a	Haplotype N ^a	A2	B2	C1	D1	Reference
Preconquest Xaltocan	Otomanguean ^b	10	10	0.300	0.300	0.000	0.400	This study
Postconquest Xaltocan	Uto-Aztecan ^b	15	15	0.600	0.200	0.067	0.133	This study
Xaltocan (all)		25	25	0.480	0.240	0.040	0.240	This study
Otomi	Otomanguean	103	68	0.466	0.233	0.233	0.068	Peñaloza-Espinosa et al., 2007; Sandoval et al., 2009
Zapotec	Otomanguean	100	72	0.410	0.240	0.300	0.050	Torrioni et al., 1994; Kemp et al., 2010
Mixtec	Otomanguean	142	65	0.684	0.211	0.070	0.035	Torrioni et al., 1994; Peñaloza-Espinosa et al., 2007; Sandoval et al., 2009; Kemp et al., 2010
Triqui	Otomanguean	107	107	0.720	0.280	0.000	0.000	Sandoval et al., 2009
Nahua-Cuetzalan	Uto-Aztecan	46	29	0.630	0.196	0.152	0.022	Malhi et al., 2003; Kemp et al., 2010
Nahua-Atocpan	Uto-Aztecan	109	44	0.431	0.376	0.147	0.046	Peñaloza-Espinosa et al., 2007; Kemp et al., 2010
Nahua-Xochimilco	Uto-Aztecan	78	35	0.743	0.167	0.090	0.000	Peñaloza-Espinosa et al., 2007; Sandoval et al., 2009
Nahua-Zitlala	Uto-Aztecan	60	14	0.733	0.233	0.017	0.017	Peñaloza-Espinosa et al., 2007; Sandoval et al., 2009
Nahua-Ixhuatlancillo	Uto-Aztecan	57	10	0.526	0.246	0.053	0.175	Peñaloza-Espinosa et al., 2007; Sandoval et al., 2009
Nahua-Necoxtla	Uto-Aztecan	62	25	0.500	0.452	0.048	0.000	Peñaloza-Espinosa et al., 2007; Sandoval et al., 2009

^a Haplogroups others than A, B, C, D, or X not included here.

^b Language affiliations for the preconquest and postconquest Xaltocan samples are hypothesized.

DNA was extracted from the researchers' saliva and buccal swab samples using the prepIT L2P kit (DNA Genotek) and the DNeasy Blood and Tissue kit (Qiagen), respectively.

PCR amplification and analysis

We performed two sets of mtDNA analyses using the aDNA samples. First, we screened the aDNA samples for the mutations that define the Native American mitochondrial haplogroups A2, B2, C1, and D1 (Schurr et al., 1990; Torrioni et al., 1993). We did not screen these samples for the RFLP that defines haplogroup X2, as this mitochondrial haplogroup has not been reported in Mesoamerican populations (Smith et al., 1999; González-Oliver et al., 2001; Schurr, 2004; Kemp et al., 2005, 2010), and all aDNA samples were identified as belonging to haplogroups A2, B2, C1, and D1. We performed the PCRs following Bolnick et al. (2012) and visualized 3 μ l of each PCR product with ethidium bromide on 6% polyacrylamide gels to confirm amplification. The RFLPs and 9-bp deletion that define haplogroups A2, B2, C1, and D1 were screened following Bolnick and Smith (2007).

To confirm haplogroup assignment and determine the haplotype of each aDNA sample, we sequenced nucleotide positions (np) 16,011–16,382 of the first hypervariable region (HVRI) of the mitochondrial genome. For this purpose, we amplified four overlapping fragments using the primers and protocol published in Bolnick et al. (2012). For samples exhibiting a cytosine (C) at np

16,189, we also amplified one additional fragment (np 16,001–16,181) using the primers published in Kemp et al. (2009). PCR products were purified using either the QIAquick PCR purification kit (Qiagen) or the combined use of magnetic carboxylate-modified microparticles (Sera-Mag) and a Biomek FX robot. We submitted the fragments to the DNA Sequencing Facility at the University of Texas at Austin for direct sequencing, with sequencing performed in both directions.

Finally, we amplified mtDNA np 15,960–16,566 from the researchers' samples using the D-loop 1 (long) primers and PCR conditions given in Kemp et al. (2010). We submitted these PCR products to the DNA Sequencing Facility at the University of Texas at Austin for purification and sequencing, with sequencing performed in both directions.

Contamination controls

aDNA studies are methodologically challenging because aDNA is usually degraded, present in small amounts, and may be obscured by contamination from exogenous sources (O'Rourke et al., 2000; Kaestle and Horsburgh, 2002; Pääbo et al., 2004; Knapp et al., 2012). To prevent and detect contamination, we followed precautions at all stages of the analysis. The archaeologists wore gloves and face masks while excavating the burials, and samples for genetic analysis were removed and wrapped in aluminum foil as soon as the burial had been properly documented. All pre-PCR work was performed in the aDNA laboratory at the University of

Texas at Austin. The aDNA laboratory is a restricted-access clean room that is dedicated to pre-PCR aDNA research and equipped with dedicated equipment, overhead UV lights, positive air pressure, and HEPA-filtered ventilation. The post-PCR laboratory was located on a separate floor of the building, and personnel movement between facilities was unidirectional (from pre-PCR aDNA lab to post-PCR lab) each day. In addition, other precautions included wearing disposable coveralls, hair covers, facemasks, sleeve covers, shoe covers, and gloves at all times in the aDNA lab, regularly decontaminating workspaces and equipment with bleach, bleaching the entire aDNA lab weekly with 3% sodium hypochlorite (50% v/v household bleach), UV irradiating the entire lab for 2 h after each use, irradiating both reagents and tubes with 254-nm UV light before use when possible, using reagents that were certified DNA-free and/or molecular grade whenever possible, performing DNA extractions and PCR setup in a laminar flow hood, using aerosol resistant filter tips in all pre-PCR analyses, treating samples with both bleach and UV radiation (254 nm) to eliminate any surface contamination, and including negative (blank) controls at all stages of the extraction and amplification process to detect any contamination that did occur. We performed at least two independent extractions (different bones and/or teeth) for each individual and confirmed our results through multiple amplifications. We also sent skeletal samples from two individuals to the aDNA laboratory at Washington State University for independent extraction and confirmation of our results. Finally, to help evaluate whether any of our results could be due to contamination during the excavations or in the laboratory, we sequenced np 15,960–16,566 of the mitochondrial genome in 11 archaeologists who participated in the Xaltocan excavations and in all researchers working in the aDNA laboratory at the University of Texas at Austin.

Statistical analyses

We performed statistical analyses to (1) characterize patterns of genetic variation at Xaltocan in preconquest and postconquest (Aztec) times, (2) determine if the preconquest and postconquest residents of the sampled households were genetically differentiated (i.e., not closely related), and (3) evaluate whether a demographic change is the most likely explanation for the observed patterns of mtDNA differentiation between the preconquest and postconquest household residents. In all analyses of the haplotype data, we excluded np 16,183 (because mutations at this site are strictly dependent on the presence of a C at np 16,189; Pfeiffer et al., 1999) and insertions in poly-C stretches (due to uncertainty in the exact position of such mutations). All statistical tests took sample size into consideration, and we considered results to be statistically significant when $P < 0.050$.

For the two temporal groups (preconquest and postconquest) and for the entire Xaltocan sample, we estimated haplogroup diversity (h), haplotype diversity (h), and nucleotide diversity (π) using the program Arlequin 3.5.1.2 (Excoffier and Lischer, 2010). Haplogroup diversity and haplotype diversity are defined as the probability that two randomly chosen haplogroups or haplotypes, respectively, are different in the population sample. Nucleotide diversity is the probability that two randomly chosen homologous nucleotide positions are different (Excoffier and Lischer, 2010). When calculating nucleotide diversity, we used the Kimura's two-parameter

model, which allows multiple substitutions at each nucleotide position as well as different substitution rates between transitions and transversions, with the transition–transversion ratio estimated from the data (Excoffier and Lischer, 2010). Haplogroup, haplotype, and nucleotide diversities for the two temporal groups were compared using two-sample t -tests (Zar, 1999).

To determine the phylogenetic relationships among haplotypes within each haplogroup, we created median-joining networks (Bandelt et al., 1999) with the software Network 4.6.1.0 (www.fluxus-engineering.com). These networks were used to illustrate the extent of haplotype sharing within and between temporal groups at Xaltocan. Network analyses also included haplotypes from modern Mesoamerican populations that speak languages belonging to the Otomanguean and Uto-Aztecan language families (Table 2) to help contextualize the haplotype variation at Xaltocan. In the networks, we identified mutations with reference to the Cambridge Reference Sequence (Anderson et al., 1981; Andrews et al., 1999). Because preliminary network constructions showed high levels of reticulation, we followed Kemp et al. (2010) in applying a default weight of 10 to all sites and downweighting polymorphic sites with higher relative mutation rates [as estimated by Meyer et al. (1999)]. Sites with more than fourfold higher rates were downweighted to four, sites with threefold higher rates were downweighted to five, and sites with twofold higher rates were downweighted to six.

To evaluate patterns of genetic differentiation at Xaltocan, we carried out two exact tests using Arlequin, with one test based on the haplogroup data and the other test based on the haplotype data. These tests evaluated the null hypothesis that the haplogroup or haplotype frequencies were identical in the preconquest and postconquest groups (indicating genetic continuity). If the preconquest and postconquest residents were genetically differentiated, the exact tests should reject the null hypothesis, indicating significantly different haplogroup and haplotype frequencies in the two temporal groups.

We also compared the two Xaltocan temporal groups and a group containing all Xaltocan samples with modern populations from Mesoamerica that speak languages belonging to the Otomanguean and Uto-Aztecan language families (Table 2). We calculated genetic distances between all population pairs by computing pairwise F_{ST} values for each haplogroup using the Kimura's two-parameter model in Arlequin and then weighted the F_{ST} values to reflect the frequencies of that haplogroup in the two populations being compared. We report weighted means of the within-haplogroup F_{ST} values [following Qamar et al. (2002)]. These values were used as variables in multidimensional scaling (MDS) analysis, performed in Systat 11 (SPSS), to generate a two-dimensional representation of the genetic differentiation among Xaltocan groups and modern Otomanguean and Uto-Aztecan populations. If the Aztec conquest was associated with demographic and genetic change at Xaltocan, there should be a substantial degree of genetic differentiation between the preconquest and postconquest Xaltocan groups.

Next, to evaluate whether the observed differences in the preconquest and postconquest samples reflect gradual change over time (rather than a sudden shift), we performed two Mantel tests to assess the correlation between genetic and temporal distances. One Mantel test treated all Xaltocan samples as a single group, while

the other treated the preconquest and postconquest Xaltocan samples as separate groups. We used the weighted means of within-haplogroup F_{ST} values as genetic distances and calculated temporal distances using the average date for each ancient group based on radiocarbon analyses (AD 1330 for the preconquest Xaltocan group, AD 1402 for the entire Xaltocan sample, and AD 1475 for the postconquest Xaltocan group) and AD 2010 for the modern populations.

We also used the software program Nsitu (Cabana, 2002; Cabana et al., 2008) to help evaluate whether genetic drift could account for the differences in mtDNA haplogroup frequencies between the preconquest and postconquest Xaltocan groups. This program uses simulations to test the null hypothesis that in situ microevolutionary processes such as genetic drift can account for the observed changes in haplogroup frequencies. If the null hypothesis is rejected (when $P < 0.050$), genetic drift alone is unlikely to account for the observed changes. Input parameters that varied in our simulations were female effective population size (N_{ef}), rate of change in population size, and number of generations between temporal groups. We tested two values for N_{ef} : 750 and 50. The first corresponds to a population with a census size of 5,000 individuals (Cabana et al., 2008), which represents our maximum estimate for the population size at Xaltocan before it was conquered (Sanders et al., 1979). The second corresponds to a census size of ~ 330 individuals, our minimum estimate. For N_{ef} , the lower value provides the more conservative test (because genetic drift can produce greater changes in haplogroup frequencies when N_{ef} is small). For change in population size, we tested the rates $-6.5\%/generation$ and $-17\%/generation$, as estimated for postconquest Xaltocan by Chimonas (2005) and Brumfiel (1991), respectively. In this case, the greater negative value represents the more conservative approach (the more population size decreases through time, the more impact genetic drift can have). Finally, we simulated one and six generations between temporal groups. We estimated the higher value using an average generation length of 27 years (Weiss, 1973; Fenner, 2005) and the dates AD 1330 and AD 1475 for the two Xaltocan groups (average dates based on radiocarbon analyses), which suggests that 5.4 generations separated the two groups. The higher value constitutes the more conservative approach here (the more generations between initial and final groups, the more time for genetic drift to change haplogroup frequencies). Our simulations were also conservative in assuming an absence of gene flow (because gene flow effectively increases breeding population sizes; Cabana et al., 2008).

Finally, we used the software BayeSSC (Excoffier et al., 2000; Anderson et al., 2005) to evaluate whether the observed genetic distance (F_{ST}) between the preconquest and postconquest Xaltocan groups was larger than expected, given that mutation and genetic drift will cause HVRI sequences to change over time even in the absence of demographic changes. This program takes a coalescence approach to generate trees using different population parameters, and summary statistics based on the simulated trees can then be compared to the observed values. Input parameters that varied in our BayeSSC simulations were female effective population size (N_{ef}) and the number of generations between temporal groups. In both cases, we used the same values as in Nsitu simulations (see above). The BayeSSC simulations assumed a constant population size over time because

TABLE 3. Genetic diversity estimates

	Haplogroup diversity (h)	Haplotype diversity (h)	Nucleotide diversity (π)
Preconquest Xaltocan	0.733	0.867	0.017
Postconquest Xaltocan	0.619	0.838	0.018
Xaltocan (all)	0.680	0.923	0.018

simulations failed to coalesce when the estimated rates of change in population size ($-6.5\%/generation$ and $-17\%/generation$) were included. In all simulations, we used the Kimura's two-parameter model and took a conservative approach by using a relatively fast mutation rate (0.0035 mutations per generation, as estimated by Howell et al., 2003). The transition–transversion ratio was estimated from our data. Each simulation was run 10,000 times.

RESULTS

We successfully recovered and analyzed DNA from 25 of the 26 individuals sampled (a success rate of 96.2%). The haplogroup and haplotype data for each individual are given in Table 1, and haplogroup frequencies for all populations included in this study are given in Table 2. Of the 25 Xaltocan samples that yielded analyzable DNA, twelve belong to haplogroup A2 (48%), six to haplogroup B2 (24%), one to haplogroup C1 (4%), and six to haplogroup D1 (24%). The observed haplogroup frequencies are consistent with the previous studies of Mesoamerican populations (Torroni et al., 1994; González-Oliver et al., 2001; Kemp et al., 2005, 2010). When the Xaltocan samples are split into two temporal groups (preconquest and postconquest residents of the sampled households), the frequencies of haplogroups A2 and C1 increase over time (from 30 to 60% and from 0 to $\sim 7\%$, respectively), whereas the frequencies of haplogroups B2 and D1 decrease over time (from 30 to 20% and from 40% to $\sim 13\%$, respectively).

Genetic diversity estimates (Table 3) indicate that the preconquest group shows significantly greater haplogroup diversity than the postconquest group ($P = 0.008$). Haplotype diversity and nucleotide diversity do not differ significantly between the two groups (haplotype diversity $P = 0.486$; nucleotide diversity $P = 0.155$).

The haplotype median-joining networks show that some haplotypes are found in multiple individuals from the same temporal groups, but the preconquest and postconquest groups exhibit distinct haplotypes. In haplogroup A2 (Fig. 2), the three preconquest samples exhibit a unique derived haplotype, whereas the postconquest Xaltocan samples exhibit two unique derived haplotypes, one derived haplotype shared with a Nahua-Cuetzalan individual, and a founding haplotype shared with one Otomí, one Nahua-Atocpan, and one Nahua-Xochimilco. In haplogroup B2 (Fig. 3), the preconquest Xaltocan samples exhibit the Native American founding haplotype, one unique derived haplotype, and one derived haplotype shared with four Otomies, one Mixtec, 10 Triquis, two Zapotecs, one Nahua-Atocpan, and one Nahua-Necoxtla. The postconquest Xaltocan samples exhibit one unique derived haplotype and one derived haplotype shared with a Mixtec individual. In haplogroup C1 (Fig. 4), the one postconquest Xaltocan sample exhibits a unique derived haplotype. In haplogroup D1 (Fig. 5), the four preconquest Xaltocan sam-

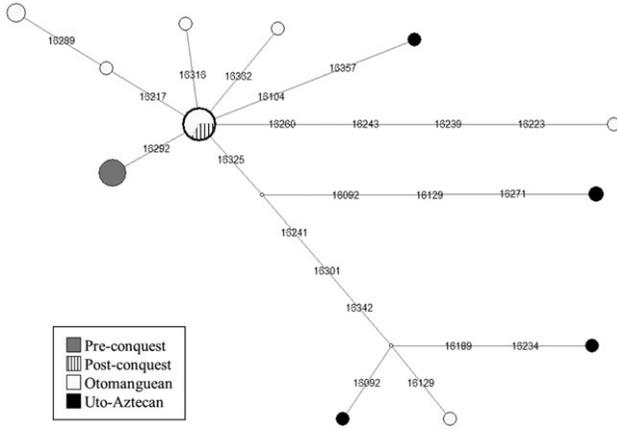


Fig. 5. Network for haplogroup D. Mutations identified with reference to the Cambridge Reference Sequence (Anderson et al., 1981; Andrews et al., 1999). Circle with a thick black line represents the founding haplotype. Circle size is proportional to the number of samples. The smallest circles represent haplotypes not found in the populations analyzed here. Information about the specific Otomanguean and Uto-Aztecan populations represented in each haplotype is available upon request.

small sample sizes and the limited power of analyzing only four variants (i.e., A–D). The exact test based on haplotype data, on the other hand, indicates that haplotype frequencies are significantly different between the preconquest and postconquest groups ($P = 0.001$). This result suggests that the null hypothesis of genetic continuity should be rejected.

Genetic distances (weighted means of within-haplogroup F_{ST} values) between populations are reported in Table 4. The MDS plot (stress value = 0.156) based on such distances (Fig. 6) shows no clear genetic differentiation between Otomanguean-speaking and Uto-Aztecan-speaking populations, but there is substantial genetic differentiation between the preconquest and postconquest Xaltocan groups. Mantel tests did not find any statistically significant correlations between genetic and temporal distances ($r = 0.414$ and $P = 0.084$ when the entire Xaltocan sample was treated as a single population; $r = 0.441$ and $P = 0.058$ when the preconquest and postconquest samples were treated as separate groups), indicating that the genetic differentiation between the preconquest and postconquest residents is not simply related to change over time.

To help evaluate whether a demographic change is the most likely explanation for the genetic differences observed between the preconquest and postconquest groups, we performed two sets of computer simulations. First, we used Nsitu to evaluate whether genetic drift could account for the observed differences in haplogroup frequencies. Although the three demographic parameters affected the degree of change in haplogroup frequencies over time, all simulations rejected the null hypothesis of genetic continuity (Table 5), suggesting that genetic drift alone cannot explain the observed differences between the preconquest and postconquest groups. Second, we used BayeSSC to evaluate whether genetic drift and mutation could explain the observed F_{ST} between the preconquest and postconquest groups. All BayeSSC simulations rejected the null hypothesis that genetic drift

TABLE 4. Weighted means of within-haplogroup F_{ST} values

	Preconquest Xaltocan	Mixtec	Zapotec	Triqui	Otomi	Postconquest Xaltocan	Nahua-Atzacapan	Nahua-Cuetzalán	Nahua-Xochimilco	Nahua-Zitlala	Nahua-Ixhuatlancillo	Nahua-Necoxtla
Mixtec	0.088											
Zapotec	0.082	0.028										
Triqui	0.281	0.089	0.084									
Otomi	0.154	0.024	0.054	0.104								
Postconquest Xaltocan	0.391	0.247	0.235	0.579	0.262							
Nahua-Atzacapan	0.082	0.040	0.016	0.078	0.050	0.225						
Nahua-Cuetzalán	0.068	0.021	0.051	0.116	0.024	0.214	0.007					
Nahua-Xochimilco	0.077	0.035	0.048	0.081	0.033	0.295	0.007	0.020				
Nahua-Zitlala	0.332	0.104	0.172	0.147	0.158	0.448	0.223	0.101	0.070			
Nahua-Ixhuatlancillo	-0.094	-0.092	-0.159	0.001	-0.132	0.209	-0.231	-0.171	-0.050	0.093		
Nahua-Necoxtla	0.098	0.100	0.136	0.304	0.161	0.315	0.092	0.107	0.158	0.380	-0.091	
Xaltocan (All)	0.041	0.151	0.138	0.386	0.164	-0.035	0.128	0.110	0.164	0.323	0.024	0.184

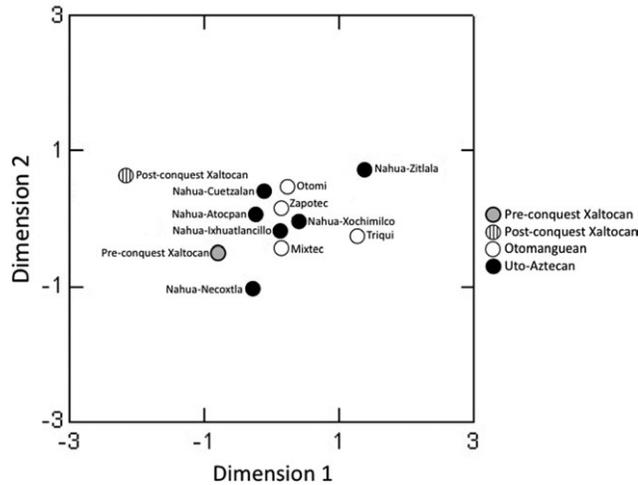


Fig. 6. MDS plot based on weighted F_{ST} values.

and mutation alone could account for the observed F_{ST} (Table 6).

DISCUSSION

Like archaeological, historical, and linguistic research, genetic studies can provide insight into the population history of the Basin of Mexico. However, accurate reconstructions of past events in the Basin of Mexico may be difficult to achieve by collecting genetic data from modern populations due to demographic complexity in the region over the last few centuries (González-José et al., 2007). aDNA studies, which provide direct characterizations of past genetic diversity, are thus especially useful for reconstructing the demographic history of pre-Hispanic populations in this region as well as the demographic consequences of events like the Tepanec and Aztec expansions. This study helps elucidate the demographic history of three households at Xaltocan by analyzing ancient mtDNA from residents of the houses in two time periods. This case study also provides insight into the demographic and genetic changes that may have occurred in Xaltocan in conjunction with the expansion of the Tepanec and Aztec states.

Unlike many previous aDNA studies investigating past demographic changes in the Americas (e.g., Kaestle and Smith, 2001; Cabana et al., 2008; Shook and Smith, 2008; Smith et al., 2009; O'Fallon and Fehren-Schmitz, 2011), which relied on samples spanning thousands of years, this study uses samples that are narrowly distributed in time. Because the preconquest and postconquest samples at Xaltocan each span only a few generations, the temporal groups defined here should accurately represent the genetic makeup of the sampled households during the two periods of interest. Furthermore, the excellent archaeological context and radiometric data for these samples permit a nuanced and fine-grained examination of the Aztec conquest and its demographic consequences for households in Xaltocan. Because 20 of the sampled individuals (from the Operation Este excavations) were buried outside two houses that were occupied across the imperial Aztec transition, we were able to directly compare the matrilineal present in these houses before and after the conquest. The other five individuals

TABLE 5. *Ns*tu results

N_{ef}	Rate of change in population size (%/generation)	Number of generations	P value
750	-6.5	1	0.004
750	-6.5	6	0.003
750	-17	1	0.002
750	-17	6	0.002
50	-6.5	1	0.002
50	-6.5	6	0.031
50	-17	1	0.002
50	-17	6	0.038

TABLE 6. *BayeSSC* results

N_{ef}	Number of generations	P value
750	1	0.001
750	6	0.002
50	1	0.015
50	6	0.042

sampled (from the Operation Y2 and Y3 excavations) were buried outside a third house in preconquest times. Although no comparative postconquest samples were available from those domestic deposits, the mtDNA data from the Y2 and Y3 samples contribute to our understanding of mitochondrial diversity at Xaltocan in preconquest times. Comparisons of the Este, Y2, and Y3 samples also permitted an initial evaluation of whether any mtDNA haplotypes were shared between households and whether any postconquest Este matrilineal may have been present in a different Xaltocan household before the Aztec conquest.

The aDNA data reported here are thought to be authentic because (1) the haplogroup and haplotype data for each individual were confirmed through multiple amplifications and multiple independent extracts, (2) stringent procedures were followed during all the stages of the analysis to prevent and detect contamination, (3) haplogroup frequencies as well as the presence of certain haplotypes accord with those previously reported in populations from the same region, (4) a subset of our results were confirmed in an independent aDNA laboratory, and (5) haplotypes of the ancient Xaltocan individuals differ from those of the archaeologists who excavated the remains and researchers working in the aDNA laboratory.

In this study, we found that multiple individuals from the same temporal group share mtDNA haplotypes, indicating close maternal relationships among those individuals. This finding is consistent with archaeological evidence suggesting that the sampled individuals represent families who occupied the Xaltocan houses over many generations. One haplotype (in haplogroup D1) is also shared between members of the preconquest Este and Y3 households, indicating that some maternally related individuals lived in different houses during the preconquest period. However, the preconquest and postconquest residents of the sampled houses exhibit very different mitochondrial patterns. Haplotype frequencies in the two temporal groups are significantly different, and each group exhibits distinct haplotypes, with the haplotypes found in different clades in the haplotype networks. Because the samples span such a short period of time from an evolutionary perspective (less than 300 years),

different recent ancestries are much more likely to account for the observed differences than the effect of genetic drift or the occurrence of new mutations. Indeed, the Nsitu and BayeSSC simulations showed that genetic drift and mutation are unlikely to account for the differences in haplogroup and haplotype composition between the preconquest and postconquest groups, suggesting that the null hypothesis of genetic continuity should be rejected. The statistical tests all rejected this null hypothesis even when the analyses were restricted to preconquest and postconquest individuals from the same households (i.e., the Este samples; data not shown). Altogether, these results are all consistent with a replacement of matriline in the sampled Xaltocan households after the Aztec conquest.

This study has relatively small sample sizes, so sampling error might have influenced the haplogroup/haplotype frequencies and patterns of haplotype sharing reported here. We note, however, that we were able to detect haplotype sharing among members of the same temporal group notwithstanding the small sample sizes. It therefore seems unlikely that the absence of haplotype sharing between the preconquest and postconquest groups is due solely to sampling error. Furthermore, we used statistical analyses that take sample size into consideration, and they found significant genetic differences between the preconquest and postconquest groups.

Genetic comparisons between the Xaltocan temporal groups and the modern Mesoamerican populations were relatively uninformative because both the haplotype networks and the MDS plot showed that there are not clear mitochondrial differences between modern Otomanguean and Uto-Aztecan populations. It is therefore possible that Postclassic Otomí and Nahuatl groups may not have been genetically distinct either. Indeed, archaeological and ethnohistorical evidence suggests that language and ethnicity may not have been closely tied to genetic ancestry in Postclassic central Mexico: at Xaltocan, for instance, it is likely that most residents stopped wearing markers of Otomí ethnicity before AD 1350 even though there is no evidence for a change in population during that time (Overholtzer, 2012). Scholars have also suggested that several Nahuatl-speaking ethnic groups were invented as a part of Aztec imperial strategies (Brumfiel, 1994; Umberger, 2008). Thus, it is not surprising that both the preconquest (Otomí) and postconquest (Aztec) residents of Xaltocan share some haplotypes with modern Otomanguean and Uto-Aztecan populations. This evidence does not mean, however, that a significant genetic discontinuity at Xaltocan cannot be detected.

Our results provide strong support for a replacement of matriline in the sampled houses that coincided with the Tepanec and Aztec conquests of Xaltocan. This finding suggests that significant demographic and genetic changes may have occurred in at least some Xaltocan households when the site was conquered, but at least three different scenarios could account for the observed genetic changes. First, the Tepanec and Aztec expansions may have led to a population replacement at Xaltocan, as 16th century documents assert. However, because the archaeological record suggests that at least some of the original Otomí population remained at Xaltocan after the defeat in 1395 and following incorporation into the Aztec empire, this scenario seems unlikely.

Second, substantial demographic change may have occurred at Xaltocan in the absence of complete population replacement. If elites and/or some commoners fled Xaltocan

after its defeat, land on the island may have become available, spurring a degree of reorganization in the community. Matrilineal changes in the sampled households may therefore reflect population movement within Xaltocan. Aztec imperialism may have also triggered an influx of commoner migrants, perhaps some sent by the Aztec ruler, and the new matriline detected in this study might represent immigrants who married members of the existing population (or descendants of the immigrants).

Third, there may have been a change in the matriline present in the sampled houses from preconquest to Aztec times, but continuity in the patriline. Sixteenth century documents suggest that central Mexican kinship in the Late Postclassic period (AD 1325–1521) was cognatic or bilateral, and houses or other property could be passed down through either the maternal or the paternal lines (Kellogg, 1988). It is therefore possible that these houses were passed down through the paternal side of the family rather than the maternal side after the Tepanec and Aztec conquests, leading to mitochondrial discontinuity but familial continuity.

Because of the limitations of this study, we cannot currently determine which of these scenarios most likely occurred. The sampled individuals were recovered from only three households and two groups of archaeological units, so the mtDNA patterns in these houses may not be representative of patterns across the entire site. The frequency of the most common haplotypes, for example, might reflect the presence of maternally related individuals in these houses rather than a high frequency of those haplotypes in the Xaltocan population as a whole. The sampling scheme in this study therefore limits our ability to determine whether a population replacement took place at Xaltocan because the replacement of matriline observed here might have been restricted to a subset of Xaltocan households. In addition, too few households were studied to determine whether the observed genetic discontinuity is due to a reorganization of Xaltocan residents and population movement within the town during the imperial Aztec transition. Nor is aDNA data from other Basin of Mexico populations available for comparison to help estimate immigration rates from sites that might have contributed migrants to Xaltocan during Aztec times. Finally, the mtDNA analyses in this study provide information about only the matrilineal history of the sampled households, so the data reported here cannot be used to evaluate whether there was continuity in the male residents of these houses from preconquest to Aztec times.

Future studies will help differentiate between the three scenarios presented here. The analysis of aDNA from individuals recovered from other houses across the site and data on a wider range of genetic markers (e.g., Y-chromosome and autosomal loci) will be very informative, and these analyses will further clarify the demographic history of Xaltocan.

CONCLUSION

This investigation constitutes the first aDNA study of how the Aztec conquest may have affected the genetic makeup of populations in the Basin of Mexico. Our results indicate that the matriline present in the sampled households at Xaltocan were replaced when the town was conquered by the Tepanecs and subsequently incorporated into the Aztec empire. Future aDNA analyses are needed to clarify the causes of the matriline

replacement observed here, but this study suggests that Aztec imperialism may have produced significant demographic and genetic changes in at least some households in Xaltocan.

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