3. An Analysis of Ancient Aztec mtDNA from Tlatelolco: Pre-Columbian Relations and the Spread of Uto-Aztecan

Brian M. Kemp, Andrés Reséndez, Juan Alberto Román Berrelleza, Ripan S. Malhi, and David Glenn Smith

Abstract: The skeletal remains of 23 of 27 Post-Classic Aztec individuals from Tlatelolco, Mexico, were found to contain analyzable mtDNA. These samples were screened for the markers that define the five founding Native American haplogroups. This skeletal collection exhibited 65.2% haplogroup A, 13% haplogroup B, 4.3% haplogroup C, and 17.4% haplogroup D. No individual's mtDNA could be assigned to haplogroup X. The haplogroup frequency distribution of this Aztec sample was compared with that of other Native American populations from the Great Basin, the American Southwest, Mesoamerica, Central America, South America, and the Caribbean. The haplogroup frequencies found in the Aztecs resemble those of other present-day Central and Southern Mexican and Central American populations, suggesting a great antiquity to this pattern of regional continuity. The data do not support a Central Mexican origin of Uto-Aztecan. Rather, they are consistent with those of earlier mtDNA studies that suggested populations from Mesoamerica had little maternal influence on the genetic structure of groups residing in the American Southwest.

Human mitochondrial DNA (mtDNA) is strictly maternally inherited (Giles et al. 1980) and is particularly useful in discerning ancestor/descendant relationships because it does not recombine during meiosis (Merriwether et al.

Biomolecular Archaeology: Genetic Approaches to the Past, edited by David M. Reed. Center for Archaeological Investigations, Occasional Paper No. 32. © 2005 by the Board of Trustees, Southern Illinois University. All rights reserved. ISBN 0-88104-089-4.

1991; Schurr et al. 1990). Rapid evolution of the mitochondrial genome (Brown et al. 1979) allows use of this molecule in studies of populations that share recent common ancestry, such as indigenous populations of the Americas.

Studies of mtDNA variation have shown that the mtDNA of most contemporary Native Americans belongs to one of five maternal lineages, designated as haplogroups A, B, C, D, and X (Brown et al. 1998; Forster et al. 1996; Schurr et al. 1990; Smith et al. 1999). Haplogroup A is defined by the gain of a *Hae*III restriction enzyme site at nucleotide position (np) 663; haplogroup B by a nine–base pair (9-bp) intergenic deletion in region V of the mtDNA genome; haplogroup C by the *Hinc*II site loss at np 13259 and an *Alu*I site gain at np 13262; haplogroup D by the *Alu*I site loss at np 5176; and haplogroup X by *Dde*I site losses at np 1715 and np 10,394 and an *Acc*I site gain at np 14465 (Brown et al. 1998; Forster et al. 1996; Schurr et al. 1990; Smith et al. 1999). Nucleotide positions correspond to those found in the Cambridge Reference Sequence (Anderson et al. 1981). Members of the same haplogroup also share polymorphisms in the control region that further define these macrolineages (Forster et al. 1996; Torroni et al. 1993). Additional variation in the control region of the molecule differentiates specific haplotypes (unique lineages) within each haplogroup. These lineages are sometimes tribal (Malhi et al. 2002; Torroni et al. 1993) or language-family specific (Derbeneva et al. 2002) and, thus, can be used to investigate fine-scale genetic relationships.

Mitochondrial DNA has been instrumental in determining the relatedness among and between native North American tribal groups (Bolnick and Smith 2003; Malhi 2001; Malhi et al. 2003, among many others), migrations into the New World (Horai et al. 1993; Lorenz and Smith 1997; Malhi et al. 2002; Merriwether and Ferrell 1996), and prehistory in the Americas (Malhi et al. 2002). Lorenz and Smith (1996) showed that in North America correlation exists between haplogroup frequencies and geographic proximity and, in some cases, language distribution. This patterning has also been revealed in the overall structure of mtDNA diversity in North America (Malhi et al. 2002).

Advances in molecular techniques, particularly the polymerase chain reaction (PCR; Saiki et al. 1988) and methods for extracting and amplifying ancient DNA (Eshleman and Smith 2001; O'Rourke et al. 2000a; Poinar et al. 1998), have made it possible to investigate DNA from pre-Columbian Native American remains. Although clear correlation can be obscured by genetic drift, as reported in the American Southeast (Bolnick and Smith 2003), studies of ancient DNA in North America have demonstrated temporal continuity of regional patterning, indicating a relatively small effect of European contact or genetic drift on the genetic structure of populations (Carlyle et al. 2000; Gonzalez-Oliver et al. 2001; Stone and Stoneking 1999; for an excellent review see O'Rourke et al. 2000b). In contrast, lack of continuity through time has been interpreted as the effect of recent migration and population replacement, such as the Numic spread into the Great Basin approximately a thousand years ago (Bettinger and Baumhoff 1982; Kaestle and Smith 2001) or as found in the northeastern United States (Schultz et al. 2001).

Interactions between the Populations of the American Southwest and Mesoamerica and the Spread of Uto-Aztecan

One area of research that can benefit from the investigation of ancient DNA is the investigation of human interactions between the American Southwest and Mesoamerica. Anthropologists have long noted the complex relationship between both past and present indigenous cultures of these regions (Foster 1996; Hedrick et al. 1974) and opinions on the topic range from the suggestion that pre-Columbian cultures of the Southwest were essentially isolated from Mesoamerican influence (Cordell 1997) to hypotheses of population movement from the latter to the former (Bellwood 2000; Hedrick et al. 1974; Hill 2001, 2002a; Matson 1999, 2002).

Some of the cultural similarities between the two regions include the introduction of Mesoamerican-style ball courts, pottery, artwork, and personal adornments to the Hohokam culture area (Cordell 1997; Haury 1974; Hedrick et al. 1974; Kelley 1995). The Anasazi cultural area also adopted Mesoamerican architectural styles, copper bells, macaws, and textile techniques (Haury 1974; Turner and Turner 1999). The connections were evidently bidirectional, because turquoise mined in the Southwest appears in Mesoamerica (Coe 1994). Furthermore, aspects of Anasazi and other Pueblo ritual, such as common deities, may have a Mesoamerican origin (Beals 1974; Parsons 1974; Turner and Turner 1999).

While the aforementioned cultural ties are intriguing, the most notable connection between the two regions is the domestication and subsequent northward spread of maize agriculture from Central Mexico (Smith 1994, 1995). Based on recent radiocarbon dates of early cultigens, the northward spread of maize occurred more rapidly than previously thought (Fritz 1994), suggesting that maize agriculture was spread via a human migration (Matson 1999) rather than via cultural diffusion.

Uto-Aztecan is one of the world's major language families, and populations that speak Uto-Aztecan languages are found in a broken chain from southern Idaho to Panama (Miller 1983). Most Uto-Aztecan scholars agree that the language family originated somewhere in the Southwest (Northern Origin Model), based on the center-of-gravity principle (Dyen 1956; Miller 1984) and biogeographical reconstruction of Proto-Uto-Aztecan (PUA) words (Fowler 1983). In contrast to the above view, Hill's (2001, 2002b) reconstruction of PUA vocabulary and structure places the origin of the language family in Central Mexico (Southern Origin Model). Hill (2001) and Bellwood (1997, 2000) hypothesize that PUA spread northward around 4500 to 3500 B.P. as a consequence of population expansion resulting from agricultural innovations. Because Uto-Aztecan is the only language family found in both regions, it has been proposed that members of this language family brought maize agriculture to the Southwest (Bellwood 2000; Hill 2001, 2002a; Matson 2002).

While the number of cultural and linguistic connections mentioned above might suggest that biological relationships between the two regions ensued as well, genetic evidence has thus far provided only equivocal support. Previous mtDNA studies of contemporary Uto-Aztecan speakers do not support the Southern Origin Model (Malhi et al. 2003; Smith et al. 2000). Smith and associates (2000)

showed that the frequencies of mtDNA haplogroups greatly differ among Uto-Aztecan speakers from Central Mexico (Nahua speakers from Cuetzalan), the Southwest (Akimel O'odham), and the Great Basin (Northern Paiute). Most populations in the Southwest exhibit high frequencies of haplogroup B and very little, if any, haplogroup A. Great Basin populations exhibit relatively high frequencies of haplogroup D (Kaestle and Smith 2001), which is rare in both the Southwest and Mesoamerica. Lastly, high frequencies of haplogroup A predominate in Mesoamerica (Malhi et al. 2003; Smith et al. 2000). An exception to this pattern is found among Athapaskan groups residing in the Southwest, who are characterized by high frequencies of haplogroup A. However, the Athapaskans are recent migrants to the Southwest from the north (Basso 1983) and carry a unique derived form of haplogroup A that is not closely related to the Mesoamerican haplogroup A (Malhi et al. 2003). Overall, this suggests minimal, if any, maternal genetic influence of southern Uto-Aztecan speakers on the gene pool of the Southwest.

Malhi and colleagues (2003) confirmed the lack of continuity in haplogroup frequencies across the two regions after increasing the sample sizes of the populations studied by Smith and colleagues (2000) and by investigating another group from the Southwest closely related to the Akimel O'odham, the Tohono O'odham. This was an important addition as the Tohono O'odham represent a tribe less likely than the Akimel O'odham to have been influenced by extratribal admixture (Brown et al. 1958), which can obscure the ancestral haplogroup frequency distribution. Malhi and colleagues (2003) also analyzed sequences from the first hypervariable segment of the control region of the mtDNA, demonstrating that nonbasal haplotypes are not shared between the two regions (Malhi et al. 2003).

In other biological studies, both support for and evidence against close genetic relations between Southwestern and Mesoamerican populations are found. In an early study of blood-group phenotypes in the Southwest and Mexico, Brown and associates (1958) suggested that Uto-Aztecan speakers of Northern Mexico and the United States are not genetically similar to the linguistically distinct groups of Central and Southern Mexico, who would presumably be related to the early Uto-Aztecan speakers responsible for the northward migration proposed by Hill (2001). Using a much larger number of protein-coding loci, Spuhler (1979) reported the same conclusion. However, Niswander and colleagues (1970) indicated that the blood-group frequencies of the Nahua, Akimel O'odham, and Tohono O'odham were similar, suggesting that these groups might be closely related to one another. The contradicting results provided by these studies might reflect sampling error by one or more of the studies, lack of rigorous statistical analyses, natural selection acting on the studied traits, or a combination of issues.

Turner (1993) analyzed 16 dental traits and suggested that no genetic link exists between Mexico and pre-Classic Hohokam, the presumed ancestors of Uto-Aztecans of the Southwest (Hale and Harris 1979). Contrary to this, another dental study that measured 29 traits clearly linked pre-Classic Ciudad Hohokam to Mexican populations (Turner 1987, 1993). Turner (1993) favors the latter results because more traits were used but admits that further investigation on Hohokam dentition is needed.

Investigation of rare albumin variants by Smith and associates (2000) showed that Albumin Mexico (AL*Mexico) is found among members of all the major

geographic branches of Uto-Aztecan, of groups speaking Yuman languages in the Southwest, and of every major language family in Mexico. Smith and associates (2000) proposed that AL*Mexico originated in Mexico prior to 3000 B.P. and spread northward into the Southwest along the Tepiman corridor.

Some of the above-cited studies suggest a nuclear DNA connection exists between Mexico and the Southwest and are in opposition to the mtDNA data. Overall, the genetic studies suggest that if Uto-Aztecan has a southern origin (Hill 2001), migrants who brought the language to the Southwest contributed only nuclear genes and little or no mtDNA. If such a population movement did occur, it must have consisted predominately of males (Malhi et al. 2003). Malhi and colleagues (2003) and Smith and colleagues (2000) have argued that other migrations in the Americas, such as the Athapaskan migration to the Southwest, were predominately of males. Another possibility could be that no migration took place, and the language family and agriculture were spread northward by cultural diffusion perhaps through the influence of merchants or explorers (sensu Anthony 1990; sensu Beaton 1991).

The Aztecs: A Brief Overview

Following the collapse of the Toltec Empire (A.D. 900–1170), the population occupying the capital city of Tula dispersed, providing the opportunity for outside immigrants to occupy Central Mexico (Townsend 1992). One such group of outsiders was the Mexica, who would one day help to reestablish order by forming the Aztec Empire. Aztec history, as revealed in codices recorded by the Spanish, attests to a humble origin of the people in a land to the north called *Atzlán*. It is unknown whether this is a true reflection of their history or a fictionalized account, for the Toltecs also claimed an origin to the north. The Aztec legend might in fact be an adoption of the Toltec story, as the Aztecs greatly respected their predecessors, copying them stylistically and architecturally (Townsend 1992).

En route to their eventual homeland, the Mexica took refuge at Tizaapan near Culhuacan, a town formed by Toltec refugees from Tula. There the Mexica intermarried with the Culhua and joined them in war against the neighboring Xochimilco. The Mexica's boasts of military prowess led the Culhua nobles, who still viewed the Mexica as barbarians, to question their presence in the region. Eventually, the sacrificial killing of the daughter of a Culhua lord by the Mexica led to their forceful expulsion from Culhuacan (Townsend 1992). The Mexica fled to one of the uninhabited islands in Lake Texcoco, on which they founded Tenochtitlan in either A.D. 1325 or 1345 (Townsend 1992) and Tlatelolco in A.D. 1337 or perhaps a few years earlier (Matos Moctezuma 1989). This marked the birthplace of the Aztec Empire.

Less than two hundred years later, the Aztec Empire collapsed at the hands of Spanish invaders led by Cortés. The population size of Tenochtitlan and Tlatelolco combined at European contact in 1519 was approximately 250,000 (Gibson 1964; Townsend 1992) but by 1560 had declined to one-fifth its former size (Gibson 1964). The Spanish instituted a program of *congregación* to control and organize the remaining indigenous populations and the newly conquered land.

This program involved the resettlement of indigenous peoples, sometime resulting in the reorganization of separate, previously unrelated tribes into new communities (Gibson 1964).

The Present Study

The current study tests for temporal continuity in Central Mexico by examining the genetic variation found in a pre-contact Aztec skeletal population from Tlatelolco. An investigation of ancient DNA from these southern Uto-Aztecan speakers addresses the question of whether mtDNA haplogroup frequencies exhibited by extant indigenous populations of Central Mexico reflect the pre-Columbian gene pool. It is possible that the paucity of detectable maternal connections between Uto-Aztecan speakers in the American Southwest and Central Mexico might be a result of genetic drift caused by population decline following European contact or the formation of *congregaciónes*. Similar reasoning has been used to explain mtDNA patterns found in the southeastern United States (Bolnick and Smith 2003). However, temporal stability of haplogroup frequencies has been demonstrated in the Southwest for the past 1,500 years (Carlyle et al. 2000; Parr et al. 1996) and for at least 1,000 years in the Yucatan (Gonzalez-Oliver et al. 2001) and is in accordance with the general trend found in North America (O'Rourke et al. 2000b). The present study also aims to characterize the relationship between the Aztecs and other Native American groups from North, Central, and South America (Table 3-1) and to further investigate the Southern Origin Model of Uto-Aztecan (Hill 2001).

Population Studied

The human remains analyzed for this study were excavated under the direction of Eduardo Matos Moctezuma in 1965 and 1966 from the ceremonial center of the Aztec island city-state of Tlatelolco, located in Lake Texcoco. The individuals chosen for the sample population are believed to be rank-and-file Aztecs, as we avoided sampling extraordinary burials or sacrificial victims. The sample population is from the Post-Classic period (defined as the tenth to sixteenth centuries A.D. in Mesoamerica), but it must postdate A.D. 1325 (or A.D. 1345), the initial founding of the island city of Tenochtitlan-Tlatelolco (Townsend 1992). Portions of these remains, which are housed in the Instituto Nacional de Antropología e Historia, Mexico City, were sent to the University of California, Davis for DNA analysis.

Ancient DNA Extraction and PCR Amplification

DNA was extracted from the remains of 27 individuals, represented by 23 ribs and 4 vertebrae, using methods specifically designed for ancient materials. At least two DNA extractions were performed on each individual. The first and second extractions from an individual took place at different times, no less than one month apart. An individual was assigned to a specific haplogroup

Table 3-1. *Mitochondrial DNA Haplogroup Frequencies Displayed by the Populations Included in This Study, Locations and Languages of the Populations, and Sources*

Population	n	%*A*	%*B*	%*C*	%*D*	%*X*	*Location*	*Language*	*Source*
Akimel O'odham	43	4.7	53.5	39.5	0	2.3	SE Arizona, N. Mexico	Uto-Aztecan	Lorenz and Smith (1996); Malhi et al. (2003); Torroni et al. (1992)
Aztecs	23	65.2	13	4.3	17.4	0	Tlatelolco	Uto-Aztecan	This study
Anasazi	25	24	60	16	0	0	American Southwest	?	Carlyle et al. (2000)
Boruca	14	21.4	71.4	0	7.1	0	Costa Rica	Chibcha-Paezan	Barrantes et al. (1990); Torroni et al. (1993)
Bribri/Cabecar	24	54.2	45.8	0	0	0	Costa Rica	Chibcha-Paezan	Barrantes et al. (1990); Torroni et al. (1993)
Cochimi	13	7.7	46.2	46.2	0	0	Baja S. Central	Yuman	Lorenz and Smith (1996); Malhi et al. (2003); Smith et al. (2000)
Cuetzalan Nahua	31	61.3	32.3	6.5	0	0	Central Mexico	Uto-Aztecan	Lorenz and Smith (1996); Malhi et al. (2003)
Delta Yuman	23	0	56.5	43.5	0	0	W. Arizona	Yuman	Lorenz and Smith (1996); Malhi et al. (2003); Smith et al. (2000)
Emberá	44	47.7	52.3	0	0	0	E. Panama	Chocó	Kolman and Bermingham (1997)
Fremont	30	0	80	13.3	6.7	0	Great Salt Lake Wetlands	?	Parr et al. (1996)
Guatuso	20	85	15	0	0	0	Costa Rica	Chibcha	Torroni et al., Mitochondrial DNA "Clock" (1994)
Guaymi/ Ngawbe/Ngöbe	62	67.7	32.3	0	0	0	W. Panama	Chibcha	Kolman et al. (1995); Spielman et al. (1979); Torroni et al. (1993)
Huetar	27	70.4	3.7	0	25.9	0	Costa Rica, Quitirrisí	Chibcha	Santos et al. (1994)

Table 3-1.—*Continued*

Population	n	*%A*	*%B*	*%C*	*%D*	*%X*	*Location*	*Language*	*Source*
Jemez	36	0	88.9	2.8	0	8.3	NW, New Mexico	Tanoan	Lorenz and Smith (1996); Malhi et al. 2003; Smith et al. (2000)
Kiliwa	7	0	100	0	0	0	Baja N. Central	Yuman	Lorenz and Smith (1996); Malhi et al. (2003); Smith et al. (2000)
Kuna	89	79.8	20.2	0	0	0	Panama	Chibcha	Barrantes et al. (1990); Batista et al. (1995); Torroni et al. (1993)
Maya ancient, Copán	9	0	0	88.9	11.1	0	Honduras	Mayan	Merriwether et al. (1997)
Maya ancient, Xcarét	24	87.5	4.2	8.3	0	0	Yucatan, Quintana Roo	Mayan	Gonzalez-Oliver et al. (2001)
Maya, contemporary	27	55.6	22.2	14.8	7.4	0	Yucatan	Mayan	Schurr et al. (1990); Torroni et al. (1992)
Mixe-Highland	16	62.5	31.3	6.2	0	0	S. Mexico, Ayutla	Mixe-Zoquean	Torroni et al., mtDNA and Y-Chromosome Polymorphisms (1994)
Mixtecs-Alta	15	73.4	13.3	13.3	0	0	S. Mexico, Nochixlan	Otomanguean	Torroni et al., mtDNA and Y-Chromosome Polymorphisms (1994)
Mixtecs-Baja	14	92.9	7.1	0	0	0	S. Mexico, Huajuapan	Otomanguean	Torroni et al., mtDNA and Y-Chromosome Polymorphisms (1994)
North Mexico	199	37.6	29.6	26.1	6.5	0	N. Mexico, Juárez and Ojinaga	?	Green et al. (2000)
Northern Paiute/ Shoshoni	94	0	42.6	9.6	47.9	0	Great Basin	Uto-Aztecan	Kaestle and Smith (2001)

Table 3-1.—*Continued*

Population	n	*%A*	*%B*	*%C*	*%D*	*%X*	*Location*	*Language*	*Source*
Pai Yuman	27	7.4	66.7	25.9	0	0	Baja North	Yuman	Lorenz and Smith (1996); Malhi et al. (2003); Smith et al. (2000)
River Yuman	22	0	63.6	36.4	0	0	W. Arizona	Yuman	Malhi et al. (2003); Smith et al. (2000); Torroni et al. (1992)
Seri	8	0	12.5	87.5	0	0	Sonora, Mexico	Seri	Malhi et al. (2002)
Tainos, ancient	24	0	0	75	25	0	Dominican Republic, La Caleta site	?	Lalueza-Fox et al. (2001)
Tohono O'odham	37	0	56.8	37.8	5.4	0	SE Arizona	Uto-Aztecan	Malhi et al. (2002)
Teribe	20	80	20	0	0	0	Upper Teribe River, Panama	Chibcha	Torroni et al., Mitochondrial DNA "Clock" (1994)
Valley Zapotecs	15	33.3	33.3	33.3	0	0	S. Mexico, Valley of Oaxaca	Otomanguean	Torroni et al., mtDNA and Y-Chromosome Polymorphisms (1994)
Wounan	31	80.6	19.4	0	0	0	E. Panama	Chocó	Kolman and Bermingham (1997)
Yanomama	24	0	16.7	54.2	29.2	0	Brazil and Venezuela	Chibcha-Paezan	Torroni et al. (1993)
Yanomama	73	0	6.8	82.1	10.9	0	Brazil	Chibcha-Paezan	Easton et al. (1996)
Yanomama	110	2.7	11	39	47.3	0	Brazil and Venezuela	Chibcha-Paezan	Merriwether et al. (2000)
Zuni	26	15.4	76.9	7.7	0	0	NW, New Mexico	Zuni	Lorenz and Smith (1996); Malhi et al. (2003)

only when at least two extractions yielded the same results. For each extraction, approximately 0.5–2.0 g of bone was removed from each sample and submerged for 10–15 minutes in 2% sodium hypochlorite (Clorox bleach diluted 1:2 with ddH_2O) to rid the bone surface of modern contaminating DNA (Kemp and Smith 2003). The bleach was then poured off, and the bone was submerged for 1–2 minutes in DNA-free ddH_2O (Gibco). After discarding the water, the bone was repeatedly rinsed with water to remove any remaining bleach.

The bones were transferred to 15 ml conical tubes and demineralized by adding a sufficient amount of 0.5 M EDTA (1–4 ml) to submerge the bones, and the tubes were then gently rocked for at least 48 hours at room temperature. Following demineralization, 3 mg of proteinase K (Gibco BRL) and 500 µl of 0.1 M of *N*-phenacylthiazolium bromide (PTB) were added to each tube. PTB has been shown to greatly decrease PCR inhibition caused by the presence of Maillard products, which can be abundant in ancient samples (Poinar et al. 1998). Samples were then incubated at 65°C for 8–14 hours to allow for maximum digestion of proteins in the bone.

DNA was extracted from the digested samples by a three-step phenol-chloroform method consisting of two extractions using an equal volume of phenol:chloroform (1:1) added to the EDTA and one extraction adding an equal volume of chloroform:isoamyl alcohol (24:1). DNA was precipitated by adding two volumes of cold absolute ethanol and one-half volume of cold 5 M ammonium acetate, then storing the solution at –20°C for 8–10 hours. The tubes were then centrifuged to pellet the DNA, the liquid was discarded, and the pellet washed 1–4 times with 80% ethanol, each time pelleting the DNA and discarding the liquid. The pellet was then dried at room temperature for 24 hours. To further remove coextracted PCR inhibitors (Kemp and Smith 2003), the pelleted DNA was resuspended in 300 µl ddH_2O and silica extracted using the Wizard DNA purification kit (Promega), following the manufacturer's instructions (except that the DNA was finally eluted with 100 µl ddH_2O).

PCR amplification reactions contained 3–5 µl DNA template, 4 µl 200 µM dNTPs, 2.5 µl 10X PCR buffer, 1.3 µl bovine serum albumin, 0.75 µl $MgCl_2$, 0.3 µl of each primer (50 µM), 1.5 units of Platinum Taq (Invitrogen), and ddH_2O to adjust the reaction to a total volume of 25 µl. Coordinates for primers used, numbered according to the Cambridge Reference Sequence (Anderson et al. 1981), are as follows: for haplogroup A, 591–611 and 765–743 (Stone and Stoneking 1993); for haplogroup B, 8195–8215 and 8316–8297 (Wrischnik et al. 1987); for haplogroup C, 13237–13256 and 13310–13290 (Handt et al. 1996); and for haplogroup D, 5099–5120 (Parr et al. 1996) and 5211–5190 (Handt et al. 1996). The samples were not tested for markers characterizing haplogroup X because all successfully extracted samples exhibited diagnostic markers defining them as belonging to haplogroups A, B, C, or D. No compound haplogroups involving haplogroup X have been reported and members of the haplogroup have not been reported in populations of Mexico, Central America, or South America. PCR conditions were as follows: 94°C for 5 minutes and 40 cycles of 30-second holds at 94°C, 55°C, and 72°C, followed by a final 3-minute extension period at 74°C. Approximately 5–7 µl of amplicon was electrophoresed on 6% polyacrylamide gels and visualized with ethidium bromide to either confirm the success-

ful amplification of mtDNA for later restriction analysis or to score the presence or absence of the 9-bp deletion (defining haplogroup B in most cases, except as described in the results section below). Restriction enzyme digestion of 7–10 µl of each amplicon was performed using 8 units of each respective enzyme (*Hae*III for haplogroup A and *Alu*I for both haplogroups C and D). These products were electrophoresed and visualized as described above.

Contamination Control

Because of the highly degraded nature of ancient DNA (Lindahl 1993; Pääbo et al. 1988), ancient samples are prone to contamination by modern DNA, and, therefore, special precautions were taken when working with the Aztec samples (according to the recommendations in Kelman and Kelman 1999). All skeletal remains and DNA extracts were prepared and stored in a positively pressured room separated from the lab in which PCR reactions took place. Access to the ancient pre-PCR lab was limited to researchers working on ancient DNA. Additionally, the first hypervariable segment of the mtDNA control region of all researchers has been sequenced (and none of them belong to haplogroups A, B, C, D, or X), allowing quick identification of modern contamination generated by laboratory personnel. Ancient DNA researchers were required to wear lab coats and disposable gloves, booties, and hairnets to help ensure a sterile environment.

All extraction procedures were performed using equipment and reagents dedicated to the ancient DNA lab. Pipetters and lab benches are routinely wiped down with 2% sodium hypochlorite. Sterile, disposable labware was used when possible and filtered, aerosol-resistant pipette tips were always used. Mock extractions and mock amplifications (in which no sample and no DNA template are added, respectively) were performed alongside every extraction and amplification, and these negative controls were electrophoresed alongside amplicons being analyzed. This allowed for the detection of the presence of contamination generated during any of the numerous steps outlined above.

Statistical Analysis

The haplogroup frequencies of the Aztecs were compared with those of other Native American groups (Table 3-1) using Fisher's exact test of homogeneity (Weir 1996), bootstrapping each comparison with 1,000 resampled iterations of the original data using the Genepop software package (Raymond and Rousset 2000). Haplogroups other than A, B, C, D, or X, reported in the literature, were not included in this analysis, as they most likely represent non–Native American admixture discovered in contemporary populations (Smith et al. 1999) or contamination detected in ancient samples.

A neighbor-joining tree with random input order was constructed in the program NEIGHBOR and visualized with DRAWTREE of the PHYLIP 3.6a2 software package (Felsenstein 1993). The GENDIST program was used to calculate genetic distances between all pairs of populations (Table 3-1) based on the chord distance measurement of Cavalli-Sforza and Edwards (1967). Malhi and

colleagues (2003) demonstrated that Yuman populations are statistically undifferentiated from each other and, therefore, in order to decrease the number of populations used in this analysis, all the Yuman-speaking groups (see Table 3-1) were grouped together as "Yuman." The Copán Maya could not be grouped with the Maya from Xcarét because of the extreme difference in haplogroup frequencies between the two populations and were excluded from the analysis because of small sample size ($n = 9$). The difference between the two Maya populations may be due to inadequate sampling in the Copán Maya (Merriwether et al. 1997), population substructure among the pre-Columbian Maya (Gonzalez-Oliver et al. 2001), or a combination of these two reasons (Gonzalez-Oliver et al. 2001).

A principal coordinates analysis was performed using the same populations as employed for constructing the neighbor-joining tree. The coordinates were calculated in GENESTAT for Windows, using genetic similarity between populations ($1–F_{ST}$), calculated from F_{ST} values determined in Arlequin version 2.000 software (Schneider et al. 2000).

Results

Of the 27 skeletal remains, 23 yielded analyzable mtDNA. The frequencies of haplogroups A, B, C, and D in this Aztec population were 65.2%, 13%, 4.3%, and 17.4%, respectively (Table 3-1). Of the four individuals for whom samples failed to yield analyzable DNA, three probably contain preserved mtDNA. That is, they revealed Native American haplogroups in one of the multiple extractions. However, because amplification results from one extraction could not be confirmed in a second independent extraction, they were excluded from the results. The fourth individual for whom results are not reported yielded no analyzable mtDNA, probably because of the overwhelming presence of PCR inhibitors coextracted with the sample, despite the measures taken to avoid such inhibition. The overall success rate is high compared with that in other studies of ancient mtDNA, probably due in part to the relatively young age of the samples and the favorable conditions for DNA preservation (Burger et al. 1999) to which the skeletal samples had been subjected.

The results of the Fisher's exact test are not shown. Of the groups investigated in this study (Table 3-1), the Aztecs are statistically indistinguishable at a 0.05 level of probability from the Guatuso, Huetar, and Teribe (Chibcha-speaking peoples), the contemporary Maya from the Yucatan, the ancient Maya from Xcarét, and the Mixtec (Otomanguean speakers); however, Aztecs are statistically different from the Nahua-speaking population from Cuetzalan.

The neighbor-joining tree (Figure 3-1), shows three geographically defined clusters: the American Southwest, Central Mexico/Central America, and South America/Caribbean. The Aztec sample lies within the Central Mexico/Central America cluster.

Fifty-seven percent of the variation in the principal coordinates analysis (Figure 3-2) is accounted for by the first coordinate (X-axis) and 20.4% is explained by the second coordinate (Y-axis). The first coordinate clearly separates the groups from the American Southwest (found on the lower left) from those of Central Mexico

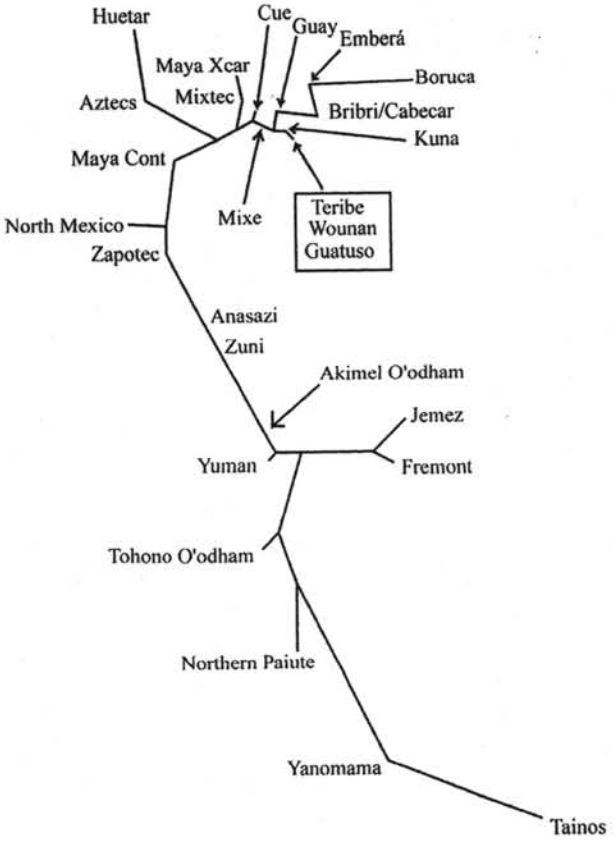

Figure 3-1. *Neighbor-joining tree constructed with chord genetic distances. Cont = Contemporary; Cue = Cuetzalan Nahua; Guay = Guaymi; Xcar = Xcarét.*

and Central America (found on the lower right). The second coordinate separates the South American and Caribbean (found on top) populations from the others.

A unique finding in this initial assessment of mtDNA variation of the Aztecs is the presence of three haplogroup A individuals who also possess the 9-bp intergenic deletion. The 9-bp deletion normally defines haplogroup B in Native Americans, but these data confirm the independent occurrence of the deletion within haplogroup A (first reported in Schurr et al. 1990). These findings are not unprecedented, as the 9-bp deletion has arisen numerous times around the world, including in Africa (Soodyall et al. 1996) and India (Watkins et al. 1999). A triplication of the 9-bp sequence has also been reported (Merriwether et al. 1995), further indicating that this region is highly subject to mutation. We confirmed that two of these three Aztec individuals are indeed members of haplogroup A, and not members of haplogroup B, by sequencing nucleotides 16250–16385 of the first hypervariable region of the control region. Both individuals revealed the characteristic haplogroup A mutations: 16290 (T), 16319 (A), and 16362 (C) relative to the mtDNA Cambridge Reference Sequence (Anderson et al. 1981).

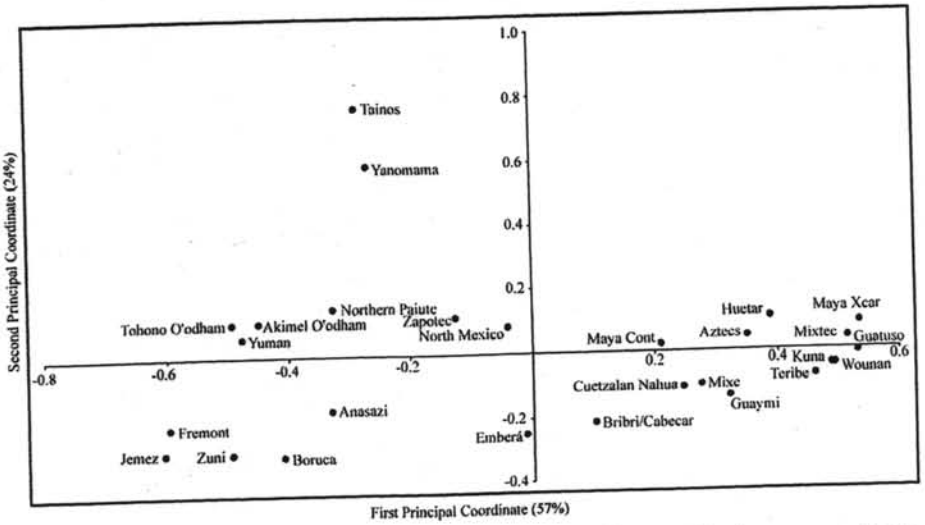

Figure 3-2. *Principal coordinates analysis. Cont = Contemporary; Xcar = Xcarét.*

Relationship of Aztecs to Other Native Americans

The Aztecs exhibit frequencies of 65.2% and 13% for haplogroups A and B, respectively, which is similar to the overall pattern found in Mexico and Central America of high frequencies of haplogroup A and lower frequencies of haplogroup B (Table 3-1). It is particularly interesting that, as in the Southwest, the haplogroup patterning cuts across both linguistic and geographic boundaries exhibiting a strong pattern of regional continuity. Speakers of Mayan, Mixe-Zoquean, Otomanguean, and Uto-Aztecan in Mexico, as well as Chibchan and Chocó speakers in Central America, all resemble one another genetically. That this pattern of regional continuity also exhibits temporal continuity is demonstrated by the Aztecs and ancient Maya from Xcarét (Gonzalez-Oliver et al. 2001). Because the pattern of haplogroup frequencies crosses geographic, linguistic, and temporal boundaries, it likely reflects a very ancient pattern. This pattern of haplogroup frequencies is quite different from those found in the Great Basin, the Southwest, South America, and the Caribbean. High frequencies of haplogroup B and low frequencies or the absence of haplogroup A characterize the Southwest and Great Basin. Populations of South America and the Caribbean exhibit high frequencies of haplogroups C and D. Both the neighbor-joining tree (Figure 3-1) and the principal coordinates analysis (Figure 3-2) illustrate these haplogroup patterns. Using only the Yanomama and an ancient Tainos population to represent populations from South America and the Caribbean, respectively, is somewhat misleading because the former population underrepresents the structure in the distribution of mtDNA haplogroups in South America (Rodriguez-Delfin et al. 2000) and, unlike findings in the latter population, a significant amount of haplogroup A has been reported in admixed populations

from Puerto Rico (Martinez-Cruzado et al. 2001). The clustering of these two populations is consistent with the hypothesis that the Caribbean was populated from South America (Lalueza-Fox et al. 2001), but it cannot rule out the possibility of some Caribbean founders originating in Mesoamerica (Martinez-Cruzado et al. 2001).

The unexpected difference between the haplogroup frequencies exhibited by the Aztecs and the modern Cuetzalan population likely stems from the presence of haplogroup D (17.4%) in the Aztecs and its corresponding absence in the Cuetzalan Nahua. This lack of continuity may be attributable to a variety of factors. First, sample sizes for both populations are reasonably small (Aztecs, $n = 23$; Cuetzalan, $n = 31$), so sampling may be responsible. Second, it is possible that Nahua-speaking populations exhibit some geographic substructure of which our samples are not representative. Third, influences of Spanish contact might also have altered the haplogroup frequencies of modern Nahua speakers, either through population decline or the establishment of the *congregaciónes*, or both, followed by genetic drift and admixture, respectively. Fourth, it is possible that the Cuetzalan population is not entirely comprised of Nahua speakers, as the blood samples were collected randomly from this city and may also include Totonac speakers (Ruben Lisker, personal communication 2002). Last, because Nahuatl was used as the lingua franca in Central Mexico at the time of European contact, genetically unrelated people might have adopted the language. Further investigation of specific haplotypes may clarify the issue.

Empire Distribution of Haplogroup A with the 9-bp Deletion

It is of particular interest that both the Aztecs and the Cuetzalan population share the derived form of haplogroup A linked with the 9-bp deletion (discussed above). Three of the 15 Aztecs who are members of haplogroup A and two of 19 haplogroup A individuals from Cuetzalan exhibited this derived form of haplogroup A (Malhi et al. 2003). This could be argued to indicate a closer relationship between the Aztecs and Cuetzalan Nahua than predicted solely by haplogroup frequencies, as has been shown by Bolnick and Smith (2003) in groups from the southeastern United States whose matrilocal populations declined drastically as a result of early European contact, which was followed by a strong influence of genetic drift on tribal haplogroup frequency distributions.

Haplogroup A linked with the 9-bp deletion has also been reported in one Boruca individual (Torroni et al. 1993), one Maya individual (Schurr et al. 1990), three Baja Mixtec (Torroni et al., mtDNA and Y-Chromosome Polymorphisms, 1994), and 10 individuals from the Northern Mexican cities of Juárez and Ojinaga (Green et al. 2000). The only report of this type outside of Mexico or Central America is in one individual from Puerto Rico (Martinez-Cruzado et al. 2001). Slave trade between the Caribbean and the Yucatan Peninsula may have brought the haplogroup A/9-bp deletion to Puerto Rico (Martinez-Cruzado et al. 2001). The Maya had strong seafaring traditions that brought them to the Caribbean (Peck 1998) and gene flow between the two areas might have occurred.

The distribution of this highly derived haplogroup A lineage crosses linguistic boundaries, occurring in Uto-Aztecan, Otomanguean, Mayan, and Chibchan speakers, which suggests that it is a very old haplotype; however, this interpretation is inconsistent with the low diversity found within the control region sequences of these haplotypes (Green et al. 2000; Malhi et al. 2003). The expansion of one or more of the series of empires of Central Mexico, involving the Toltecs, Aztecs, or both, might be responsible for distributing this haplotype to its present-day locations. Since the Aztecs intermarried with remnant Toltec populations before settling at Tenochtitlan, it will be difficult to assign this responsibility to one or the other empire. The Toltecs and Aztecs had known connections with at least some of the groups containing this haplotype. To begin with, there is evidence that cultural connections were made between the Toltecs and the Maya (Townsend 1992), which might have led to admixture as well. The Aztecs conquered the Mixtecs and persisted in extracting tribute from them (Townsend 1992). It is of interest that the lowland (Baja) Mixtec population contains the haplogroup A/9-bp deletion, while this haplotype has not been reported in the highland (Alta) Mixtec (Torroni et al., mtDNA and Y-Chromosome Polymorphisms, 1994). If the Aztecs introduced this haplotype into the Mixtec population it would most likely have been to the lowland population (Baja), not the populations residing in the highlands, as they would have been more difficult to contact. However, this picture may be obscured by small sample sizes representing the Alta and Baja Mixtec ($n = 15$ and $n = 14$, respectively). The one Boruca individual (Torroni et al. 1993) with this type is more difficult to explain because the Boruca, who reside in Costa Rica (Barrantes et al. 1990), are not known to us to have come into direct contact with either the Toltecs or the Aztecs. However, Nahua speakers are found in Costa Rica, having relocated there after the collapse of the Toltec Empire when tribes spread south and east from Mexico (Lothrop 1926). In addition, Aztec trading and raiding parties followed routes through Costa Rica, traveling as far south as Panama (Lothrop 1926). Therefore, introduction of this haplotype into the Boruca would not necessarily involve long-distance gene flow. It is also difficult to explain the presence of this haplotype in the Northern Mexican populations from Juárez and Ojinaga because their ethnic affiliation was not reported (Green et al. 2000). The study of Green and associates (2000) that reported the presence of this haplotype assessed the degree of European and African admixture in two cosmopolitan Mexican cities and did not focus on population prehistory. Although some of the samples from Ojinaga came from the indigenous peoples of the area, it is not known whether these particular individuals carried the derived form of haplogroup A (Lance Green, personal communication 2002).

The influence of empire building on the distribution of this haplotype requires further testing. Generating sequence data for all individuals carrying the haplotype would aid in locating its possible origin, and estimating the true amount of diversity found within this highly derived sub-clade of haplogroup A would provide a measure of its relative antiquity. If it proves to be old, a dissemination of the haplotype by an empire need not be invoked. Ancient DNA studies of the preceding empires (e.g., Toltec and Teotihuacan) might also reveal whether its origin is local or, alternatively, located outside of Central Mexico.

It is also possible that the true distribution of the haplogroup A/9-bp deletion has been obscured if other studies of mtDNA from Mexico and Central America have overlooked this unique genetic marker, either disregarding the result as contamination or categorizing it as haplogroup "other."

The Southern Origin of Uto-Aztecan

Hill (2001) attributed the northward spread of Uto-Aztecan to a population expansion of early agriculturalists from Mesoamerica around 4500–3500 B.P. The Southern Origin Model for Uto-Aztecan (Hill 2001) is not supported by the Aztec mtDNA evidence. Our data confirm the results of previous studies (Malhi et al. 2003; Smith et al. 2000), suggesting that Mesoamerican populations had little or no maternal influence on the mtDNA gene pool of the American Southwest. High frequencies of haplogroup A and low frequencies of haplogroup B characterize all groups in Mesoamerica, while most groups in the Southwest (except Athapaskans) are characterized by high frequencies of haplogroup B and low frequencies or the absence of haplogroup A. This genetic differentiation is clearly illustrated by the neighbor-joining tree (Figure 3-1) and the principal coordinates analysis (Figure 3-2), in which the Uto-Aztecan speakers from Mesoamerica (Aztecs and Cuetzalan Nahua) are well separated from Uto-Aztecan speakers to the north (Northern Paiute, Akimel O'odham, and Tohono O'odham).

It is possible, however, that Uto-Aztecan originated in the south and was brought to the Southwest by a migration made up primarily of men (Malhi et al. 2003; Smith et al. 2000). Evidence for this comes from the detection of the rare albumin variant AL*Mexico in populations of both regions (Smith et al. 2000), some similarities in dental morphology (Turner 1987, 1993), frequencies of blood-group phenotypes (Niswander et al. 1970), and genetic evidence that other migrations in North America were male dominated (Malhi et al. 2003; Smith et al. 2000).

Hill's (2001) model also suggests that the founders of the Aztec Empire did not originate far to the north but rather migrated to the Central Valley of Mexico from the northwest quadrant of Central Mexico. The mtDNA haplogroup frequencies exhibited by the Aztecs support this notion, as they resemble those of other groups from Central Mexico. Thus, the Aztec haplogroup frequencies reported here might be a valid representation of a much older mtDNA pattern characteristic of the whole of Mesoamerica. If, alternatively, the Aztec legend of a northern origin of their people is correct and they migrated into Central Mexico relatively recently (within the past eight hundred years) before establishing themselves in the Valley of Mexico (Townsend 1992), how did they come to genetically resemble their linguistically unrelated neighboring groups (i.e., Otomanguean, Mayan, and Mixe-Zoquean speakers)? Intermarriage with the Toltecs and later interactions with surrounding groups (Lothrop 1926; Townsend 1992) might have caused the loss of an Aztec mtDNA signature (if one ever existed). Generating control region sequence data for the Aztecs might aid in resolving this issue.

Conclusions

The mtDNA haplogroups of 23 of 27 Aztec samples demonstrate their similarity to other Central Mexican and Central American populations and dissimilarity with other populations including Uto-Aztecan speakers from the U.S. Southwest. Failure of the Aztec haplogroup frequency distribution to statistically resemble that of the Cuetzalan Nahua might result from sampling error, population decline, or unknown demographic or genetic events, such as population substructure or admixture. The presence of the rare haplogroup A/9-bp deletion haplotype has been discovered in both the Aztecs and Cuetzalan Nahua, suggesting closer genetic ties than demonstrated solely in haplogroup comparison. We hypothesize that one of the many Central Mexican empires, possible the Toltecs or the Aztecs, or both, spread this genetic marker to the peripheral groups in which it is found today. Our data do not fully support the Southern Origin Model of Uto-Aztecan (Hill 2001) but rather support previous studies that indicate Mesoamerican populations had little maternal genetic influence on populations of the Southwest (Malhi et al. 2003; Smith et al. 2000). Future studies of control region sequence variation and Y-chromosome variation of the Aztecs and other Mesoamerican populations will greatly aid in understanding the relationship between the Aztecs and their neighbors and help elucidate the history of the Aztec Empire.

Acknowledgments

We thank Cara Monroe for helpful comments on the essay. This work was supported by NIH grant RR00169 and a faculty research grant from the regents of the University of California.

References

Anderson, S., A. T. Bankier, B. G. Barrell, M. H. L. de Bruijn, A. R. Coulson, J. Drouin, I. C. Eperon, D. P. Nierlich, B. A. Roe, F. Sanger, P. H. Schreier, A. J. H. Smith, R. Staden, and I. G. Young
1981 Sequence and Organization of the Human Mitochondrial Genome. *Nature* 290:457–465.

Anthony, David W.
1990 Migration in Archeology: The Baby and the Bathwater. *American Anthropologist* 92:895–914.

Barrantes, Ramiro, Peter E. Smouse, Harvey W. Mohrenweiser, Henry Gershowitz, Jorge Azofeifa, Tomas D. Arias, and James V. Neel
1990 Microevolution in Lower Central America: Genetic Characterization of the Chibcha-Speaking Groups of Costa Rica and Panama, and a Consensus Taxonomy Based on Genetic and Linguistic Affinity. *American Journal of Human Genetics* 46(1):63–84.

Basso, Keith H.
1983 Western Apache. In *Southwest*, edited by A. Ortiz, pp. 139–152. Handbook of

North American Indians, Vol. 10, W. C. Sturtevant, general editor, Smithsonian Institution, Washington, D.C.

Batista, Oriana, Connie J. Kolman, and Eldredge Bermingham
1995 Mitochondrial DNA Diversity in the Kuna Amerinds of Panama. *Human Molecular Genetics* 4(5):921–929.

Beals, Ralph L.
1974 Cultural Relations between Northern Mexico and the Southwest United States: Ethnologically and Archaeologically. In *The Mesoamerican Southwest: Readings in Archaeology, Ethnohistory, and Ethnology*, edited by C. L. Riley, pp. 52–57. Southern Illinois University Press, Carbondale. First published in *El norte de México y el sur de Estados Unidos, Tercera Reunión de Mesa Redonda sobre Problemas Antropológicos de México y Cento América* (1943), pp. 191–199.

Beaton, John M.
1991 Colonizing Continents: Some Problems from Australia and the Americas. In *The First Americans: Search and Research*, edited by D. J. Meltzer, pp. 209–230. CRC Press, Boca Raton.

Bellwood, Peter
1997 Prehistoric Cultural Explanations for Widespread Language Families. In *Archaeology and Linguistics: Aboriginal Australia in Global Perspectives*, edited by P. McConrell and N. Evans, pp. 123–134. Oxford University Press, Melbourne.
2000 The Time Depth of Major Language Families: An Archaeologist's Perspective. In *Time Depth in Historical Linguistics*, Vol. 1, edited by C. Renfrew, A. McMahon, and R. L. Trask, pp. 109–140. McDonald Institute for Archaeological Research, Cambridge, England.

Bettinger, Robert L., and Martin A. Baumhoff
1982 The Numic Spread: Great Basin Cultures in Competition. *American Antiquity* 47:485–503.

Bolnick, Deborah A., and David G. Smith
2003 Unexpected Patterns of Mitochondrial DNA Variation among Native Americans from the Southeastern United States. *American Journal of Physical Anthropology* 122(4):336–354.

Brown, K. S., B. L. Hanna, A. A. Dahlberg, and H. H. Strandskov
1958 The Distribution of Blood Group Alleles among Indians of Southwest North America. *American Journal of Human Genetics* 10:175–195.

Brown, Michael D., Seyed H. Hosseini, Antonio Torroni, Hans-Jürgen Bandelt, Jon C. Allen, Theodore G. Schurr, Rosaria Scozzari, Fulvio Cruciani, and Douglas C. Wallace
1998 mtDNA Haplogroup X: An Ancient Link between Europe/Western Asia and North America? *American Journal of Human Genetics* 63(6):1852–1861.

Brown, Wesley M., Matthew George, Jr., and Allan C. Wilson
1979 Rapid Evolution of Animal Mitochondrial DNA. *Proceedings of the National Academy of Sciences of the United States of America* 74(4):1967–1971.

Burger, Joachim, Susanne Hummel, Bernd Herrmann, and Winfried Henke
1999 DNA Preservation: A Microsatellite-DNA Study on Ancient Skeletal Remains. *Electrophoresis* 20(8):1722–1728.

Carlyle, Shawn W., Ryan L. Parr, M. Geoffrey Hayes, and Dennis H. O'Rourke
2000 Context of Maternal Lineages in the Greater Southwest. *American Journal of Physical Anthropology* 113(1):85–101.

Cavalli-Sforza, Luigi Luca, and Anthony W. F. Edwards
1967 Phylogenetic Analysis Models and Estimation Procedures. *American Journal of Human Genetics* 19:223–257.

Coe, Michael D.
1994 *Mexico: From the Olmecs to the Aztecs.* 4th ed. Thames and Hudson, New York.
Cordell, Linda S.
1997 *Archaeology of the Southwest.* 2d ed. Academic Press, San Diego.
Derbeneva, Olga A., Rem I. Sukernik, Natalia V. Volodko, Seyed H. Hosseini, Marie T. Lott, and Douglas C. Wallace
2002 Analysis of Mitochondrial DNA Diversity in the Aleuts of the Commander Islands and Its Implications for the Genetic History of Beringia. *American Journal of Human Genetics* 71:415–421.
Dyen, Isidore
1956 Language Distribution and Migration Theory. *Language* 32:611–626.
Easton, Ruth D., D. Andrew Merriwether, Douglas E. Crews, and Robert E. Ferrell
1996 mtDNA Variation in the Yanomami: Evidence for Additional New World Founding Lineages. *American Journal of Human Genetics* 59(1):213–225.
Eshleman, Jason, and David Glenn Smith
2001 Use of DNase to Eliminate Contamination in Ancient DNA Analysis. *Electrophoresis* 22(20):4316–4319.
Felsenstein, Joe
1993 PHYLIP (Phylogeny Inference Package). 3.6a2 ed. Department of Genetics, University of Washington, Seattle.
Forster, Peter, Rosalind Harding, Antonio Torroni, and Hans-Jürgen Bandelt
1996 Origin and Evolution of Native American mtDNA Variation: A Reappraisal. *American Journal of Human Genetics* 59(4):935–945.
Foster, Michael K.
1996 Language and the Culture History of North America. In *Languages,* edited by I. Goddard, pp. 64–110. Handbook of North American Indians, Vol. 17, W. C. Sturtevant, general editor, Smithsonian Institution, Washington, D.C.
Fowler, Catherine S.
1983 Some Lexical Clues to Uto-Aztecan Prehistory. *International Journal of American Linguistics* 49(3):224–257.
Fritz, Gayle J.
1994 Are the First American Farmers Getting Younger? *Current Anthropology* 35(3): 305–309.
Gibson, Charles
1964 *The Aztecs under Spanish Rule: A History of the Indians of the Valley of Mexico, 1519–1810.* Stanford University Press, Stanford.
Giles, Richard E., Hugues Blanc, Howard M. Cann, and Douglas C. Wallace
1980 Maternal Inheritance of Human Mitochondrial DNA. *Proceedings of the National Academy of Sciences of the United States of America* 77(11):6715–6719.
González-Oliver, Angélica, Lourdes Márquez-Morfin, José C. Jiménez, and Alfonso Torre-Blanco
2001 Founding Amerindian Mitochondrial DNA Lineages in Ancient Maya from Xcarét, Quintana Roo. *American Journal of Physical Anthropology* 116(3):230–235.
Green, Lance D., James N. Derr, and Alec Knight
2000 mtDNA Affinities of the Peoples of North-Central Mexico. *American Journal of Human Genetics* 66(3):989–998.
Hale, Kenneth, and David Harris
1979 Historical Linguistics and Archeology. In *Southwest,* edited by A. Ortiz, pp. 170–177. Handbook of North American Indians, Vol. 9, W. C. Sturtevant, general editor, Smithsonian Institution, Washington, D.C.

Handt, Oliva, Matthias Krings, Ryk H. Ward, and Svante Pääbo
1996 The Retrieval of Ancient Human DNA Sequences. *American Journal of Human Genetics* 59(2):368–376.

Haury, Emil W.
1974 The Problem of Contacts between the Southwestern United States and Mexico. In *The Mesoamerican Southwest: Readings in Archaeology, Ethnohistory, and Ethnology,* edited by C. L. Riley, pp. 92–102. Southern Illinois University Press, Carbondale. First published in *Southwestern Journal of Anthropology* 1(1945):55–74.

Hedrick, Basil Calvin, J. Charles Kelley, and Carroll L. Riley
1974 *The Mesoamerican Southwest: Readings in Archaeology, Ethnohistory, and Ethnology.* Southern Illinois University Press, Carbondale.

Hill, Jane H.
2001 Proto-Uto-Aztecan: A Community of Cultivators in Central Mexico? *American Anthropologist* 103(4):913–934.
2002a The Origins of the Uto-Aztecans. Paper presented at the 5th Coloquio Paul Kirchoff "Desiertos y fronteras, una lectura anthropológica de la historia," Mexico City.
2002b Proto-Uto-Aztecan Cultivation and the Northern Devolution. In *Examining the Farming/Language Dispersal Hypothesis,* edited by P. Bellwood and C. Renfrew, pp. 331–340. McDonald Institute for Archaeological Research, Cambridge, England.

Horai, Satoshi, Rumi Kondo, Yuko Nakagawa-Hattori, Seiji Hayashi, Shunro Sonoda, and Kazuo Tajima
1993 Peopling of the Americas, Founded by Four Major Lineages of Mitochondrial DNA. *Molecular Biology and Evolution* 10(1):23–47.

Kaestle, Frederika A., and David Glenn Smith
2001 Ancient Mitochondrial DNA Evidence for Prehistoric Population Movement: The Numic Expansion. *American Journal of Physical Anthropology* 115(1):1–12.

Kelley, J. Charles
1995 Trade Goods, Traders and Status in Northwestern Greater Mesoamerica. In *The Gran Chichimeca: Essays on the Archaeology and Ethnohistory of Northern Mesoamerica,* edited by J. E. Reyman, pp. 102–145. Avebury, Aldershot, Great Britain.

Kelman, Lori M., and Zvi Kelman
1999 The Use of Ancient DNA in Paleontological Studies. *Journal of Vertebrate Paleontology* 19(1):8–20.

Kemp, Brian M., and David Glenn Smith
2003 Tackling (Some of) the Vagaries of aDNA Work. Paper presented at the 72nd Annual Meeting of the American Association of Physical Anthropology, Tempe, Arizona.

Kolman, Connie J., and Eldredge Bermingham
1997 Mitochondrial and Nuclear DNA Diversity in the Choco and Chibcha Amerinds of Panamá. *Genetics* 147(3):1289–1302.

Kolman, Connie J., Eldredge Bermingham, Richard Cooke, Ryk H. Ward, Tomas D. Arias, and Françoise Guionneau-Sinclair
1995 Reduced mtDNA Diversity in the Ngobe Amerinds of Panama. *Genetics* 140(1):275–283.

Lalueza-Fox, C., F. Luna Calderon, F. Calafell, B. Morera, and J. Bertranpetit
2001 mtDNA from Extinct Tainos and the Peopling of the Caribbean. *Annals of Human Genetics* 65(2):137–151.

Lindahl, Tomas
1993 Instability and Decay of the Primary Structure of DNA. *Nature* 362:709–715.

Lorenz, Joseph G., and David Glenn Smith
1996 Distribution of Four Founding mtDNA Haplogroups among Native North Americans. *American Journal of Physical Anthropology* 101(3):307–323.
1997 Distribution of Sequence Variation in the mtDNA Control Region of Native North Americans. *Human Biology* 69(6):749–776.

Lothrop, Samuel K.
1926 *Pottery of Costa Rica and Nicaragua.* Contributions from the Museum of the American Indian, Heye Foundation, Vol. 8. Museum of the American Indian Heye Foundation, New York.

Malhi, Ripan S.
2001 Investigating Prehistoric Population Movements in North America with Ancient and Modern DNA. Unpublished Ph.D. dissertation, Department of Anthropology, University of California, Davis.

Malhi, Ripan S., Jason A. Eshleman, Jonathan A. Greenberg, Deborah A. Weiss, Beth A. Schultz Shook, Frederika A. Kaestle, Joseph G. Lorenz, Brian M. Kemp, John R. Johnson, and David Glenn Smith
2002 The Structure of Diversity within New World Mitochondrial DNA Haplogroups: Implications for the Prehistory of North America. *American Journal of Human Genetics* 70(4):905–919.

Malhi, Ripan S., Holly M. Mortensen, Jason A. Eshleman, Brian M. Kemp, Joseph G. Lorenz, Frederika A. Kaestle, John R. Johnson, Clara Gorodezky, and David Glenn Smith
2003 Native American mtDNA Prehistory in the American Southwest. *American Journal of Physical Anthropology* 120:108–124.

Martinez-Cruzado, J. C., G. Toro-Labrador, V. Ho-Fung, M. A. Estevez-Montero, A. Lobaina-Manzanet, D. A. Padovani-Claudio, H. Sanchez-Cruz, P. Ortiz-Bermudez, and A. Sanchez-Crespo
2001 Mitochondrial DNA Analysis Reveals Substantial Native American Ancestry in Puerto Rico. *Human Biology* 73(4):491–511.

Matos Moctezuma, Eduardo
1989 *The Aztecs.* Rizzoli, New York.

Matson, R. G.
1999 The Spread of Maize to the Colorado Plateau. *Archaeology Southwest* 13(1):10–11.
2002 The Spread of Maize Agriculture in the U.S. Southwest. In *Examining the Farming/Language Dispersal Hypothesis*, edited by P. Bellwood and C. Renfrew, pp. 331–340. McDonald Institute for Archaeological Research, Cambridge, England.

Merriwether, D. Andrew, Andrew G. Clark, Scott W. Ballinger, Theodore G. Schurr, Himla Soodyall, Trefor Jenkins, Stephen T. Sherry, and Douglas C. Wallace
1991 The Structure of Human Mitochondrial DNA Variation. *Journal of Molecular Evolution* 33(6):543–555.

Merriwether, D. Andrew, and Robert E. Ferrell
1996 The Four Founding Lineage Hypothesis for the New World: A Critical Reevaluation. *Molecular Phylogenetics and Evolution* 5(1):241–246.

Merriwether, D. Andrew, Brian M. Kemp, Douglas E. Crews, and James V. Neel
2000 Gene Flow and Genetic Variation in the Yanomama as Revealed by Mitochondrial DNA. In *America Past, America Present: Genes and Languages in the Americas and Beyond*, edited by C. Renfrew, pp. 89–124. McDonald Institute for Archaeological Research, Cambridge, England.

Merriwether, D. Andrew, David M. Reed, and Robert E. Ferrell
1997 Ancient and Contemporary Mitochondrial DNA Variation in the Maya. In

Bones of the Maya: Studies of Ancient Skeletons, edited by S. L. Whittington and D. M. Reed, pp. 208–217. Smithsonian Institution Press, Washington, D.C.

Merriwether, D. Andrew, Francisco Rothhammer, and Robert E. Ferrell
1995 Distribution of the Four Founding Lineage Haplotypes in Native Americans Suggests a Single Wave of Migration for the New World. *American Journal of Physical Anthropology* 98(4):411–430.

Miller, Wick R.
1983 Uto-Aztecan Languages. In *Southwest*, edited by A. Ortiz, pp. 113–124. Handbook of North American Indians, Vol. 10, W. C. Sturtevant, general editor, Smithsonian Institution, Washington, D.C.
1984 The Classification of the Uto-Aztecan Languages Based on Lexical Evidence. *International Journal of American Linguistics* 50(1):1–24.

Niswander, J. D., K. S. Brown, B. Y. Iba, W. C. Leyshon, and P. L. Workman
1970 Population Studies on Southwestern Indian Tribes. I: History, Culture, and Genetics of the Papago. *American Journal of Human Genetics* 22:7–23.

O'Rourke, Dennis H., M. Geoffrey Hayes, and Shawn W. Carlyle
2000a Ancient DNA Studies in Physical Anthropology. *Annual Review of Anthropology* 29:217–242.
2000b Spatial and Temporal Stability of mtDNA Haplogroup Frequencies in Native North America. *Human Biology* 72(1):15–34.

Pääbo, Svante, John A. Gifford, and Allan C. Wilson
1988 Mitochondrial DNA Sequences from a 7000-Year Old Brain. *Nucleic Acids Research* 16(20):9775–9787.

Parr, Ryan L., Shawn W. Carlyle, and Dennis H. O'Rourke
1996 Ancient DNA Analysis of Fremont Amerindians of the Great Salt Lake Wetlands. *American Journal of Physical Anthropology* 99(4):507–518.

Parsons, Elsie Clews
1974 Some Aztec and Pueblo Parallels. In *The Mesoamerican Southwest: Readings in Archaeology, Ethnohistory, and Ethnology*, edited by C. L. Riley, pp. 131–146. Southern Illinois University Press, Carbondale. First published in *American Anthropologist* 35(1933):611–631.

Peck, Douglas T.
1998 The Case for Prehistoric Cultural Contact between the Maya on the Yucatán and the Indians of Florida. *Florida Anthropologist* 51(1):3–14.

Poinar, Hendrik N., Michael Hofreiter, W. Geoffrey Spaulding, Paul S. Martin, B. Artur Stankiewicz, Helen Bland, Richard P. Evershed, Goran Possnert, and Svante Pääbo
1998 Molecular Coproscopy: Dung and Diet of the Extinct Ground Sloth *Nothrotheriops shastensis*. *Science* 281(5375):402–406.

Raymond, Michel, and Francois Rousset
2000 Genepop on the Web. Population Differentiation Option.Electronic document, http://wbiomed.curtin.edu.au/genepop, accessed May 28, 2002.

Rodriguez-Delfin, Luis A., Veronica E. Rubin-de-Celis, and Marco A. Zago
2000 Genetic Diversity in an Andean Population from Peru and Regional Migration Patterns of Amerindians in South America: Data from Y Chromosome and Mitochondrial DNA. *Human Heredity* 51(1-2):97–106.

Saiki, Randall K., David H. Gelfand, Susanne Stoffel, Stephen J. Scharf, Russell Higuchi, Glenn T. Horn, Kary B. Mullis, and Henry A. Erlich
1988 Primer-Directed Enzymatic Amplification of DNA with a Thermostable DNA Polymerase. *Science* 239:487–491.

Santos, Maria, Ryk H. Ward, and Ramiro Barrantes
1994 mtDNA Variation in the Chibcha Amerindian Huetar from Costa Rica. *Human Biology* 66(6):963–977.
Schneider, Stefan, David Roessli, and Laurent Excoffier
2000 ARLEQUIN ver. 2.000: A Software for Population Genetic Analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland. Electronic document, http://anthro.unige.ch/arlequin, accessed May 28, 2002.
Schultz, Beth A., Ripan S. Malhi, and David Glenn Smith
2001 Examining the Proto-Algonquian Migration: Analysis of mtDNA. In *Papers of the 32nd Algonquian Conference*, edited by J. D. Nichols, pp. 470–492. University of Manitoba, Winnipeg.
Schurr, Theodore G., Scott W. Ballinger, Yik-Yuen Gan, Judith A. Hodge, D. Andrew Merriwether, Dale N. Lawrence, William C. Knowler, Kenneth M. Weiss, and Douglas 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(3):613–623.
Smith, Bruce D.
1994 The Origins of Agriculture in the Americas. *Evolutionary Anthropology* 3(5):174–184.
1995 *The Emergence of Agriculture*. Scientific American Library, New York.
Smith, David Glenn, Joseph Lorenz, Becky K. Rolfs, Robert L. Bettinger, Brian Green, Jason Eshleman, Beth Schultz, and Ripan Malhi
2000 Implications of the Distribution of Albumin Naskapi and Albumin Mexico for New World Prehistory. *American Journal of Physical Anthropology* 111(4):557–572.
Smith, David Glenn, Ripan S. Malhi, Jason Eshleman, Joseph G. Lorenz, and Frederika A. Kaestle
1999 Distribution of mtDNA Haplogroup X among Native North Americans. *American Journal of Physical Anthropology* 110(3):271–284.
Soodyall, Himla, Linda Vigilant, Adrian V. Hill, Mark Stoneking, and Trefor Jenkins
1996 mtDNA Control-Region Sequence Variation Suggests Multiple Independent Origins of an "Asian-Specific" 9-bp Deletion in Sub-Saharan Africans. *American Journal of Human Genetics* 58(3):595–608.
Spielman, Richard S., Ernest C. Migliazza, James V. Neel, Henry Gershowitz, and Reina Torres de Arauz
1979 The Evolutionary Relationships of Two Populations: A Study of the Guaymi and the Yanomama. *Current Anthropology* 20(2):377–388.
Spuhler, James N.
1979 Genetic Distances, Trees, and Maps of North American Indians. In *The First Americans: Origins, Affinities, and Adaptations*, edited by A. B. Harper, pp. 135–184. Gustav Fischer, New York.
Stone, Anne C., and Mark Stoneking
1993 Ancient DNA from a Pre-Columbian Amerindian Population. *American Journal of Physical Anthropology* 92(4):463–471.
1999 Analysis of Ancient DNA from a Prehistoric Amerindian Cemetery. *Philosophical Transactions of the Royal Society of London B Biological Sciences* 354(1379):153–159.
Torroni, Antonio, Yu-Sheng Chen, Ornella Semino, Augusta-Silvana Santachiara-Benerecetti, C. Ronald Scott, Marie T. Lott, Marcus Winter, and Douglas C. Wallace
1994 mtDNA and Y-Chromosome Polymorphisms in Four Native American Populations from Southern Mexico. *American Journal of Human Genetics* 54(2):303–318.

Torroni, Antonio, James V. Neel, Ramiro Barrantes, Theodore G. Schurr, and Douglas C. Wallace
1994 Mitochondrial DNA "Clock" for the Amerinds and Its Implications for Timing Their Entry into North America. *Proceedings of the National Academy of Sciences of the United States of America* 91(3):1158–1162.

Torroni, Antonio, Theodore G. Schurr, Margaret F. Cabell, Michael D. Brown, James V. Neel, Merethe Larsen, David G. Smith, Carlos M. Vullo, and Douglas C. Wallace
1993 Asian Affinities and Continental Radiation of the Four Founding Native American mtDNAs. *American Journal of Human Genetics* 53(3):563–590.

Torroni, Antonio, Theodore G. Schurr, Chi-Chuan Yang, Emöke J. E. Szathmary, Robert C. Williams, Moses S. Schanfield, Gary A. Troup, William C. Knowler, Dale N. Lawrence, Kenneth M. Weiss, and Douglas C. Wallace
1992 Native American Mitochondrial DNA Analysis Indicates That the Amerind and the Nadene Populations Were Founded by Two Independent Migrations. *Genetics* 130:153–162.

Townsend, Richard F.
1992 *The Aztecs.* Thames and Hudson, London.

Turner, Christy G. II
1987 Affinity and Dietary Assessment of Hohokam Burials from the Site of La Cuidad, Central Arizona. In *La Ciudad: Specialized Studies in the Economy, Environment, and Culture of La Ciudad, Parts I and II,* edited by J. E. Kisselburg, G. E. Rice, and B. L. Shears, pp. 215–230. Anthropological Field Studies, Arizona State University, Tempe.
1993 Southwest Indian Teeth. Southwest Indians: Prehistory through Dentition. *National Geographic Research and Exploration* 9(1):32–53.

Turner, Christy G. II, and Jacqueline A. Turner
1999 *Man Corn: Cannibalism and Violence in the Prehistoric American Southwest.* University of Utah Press, Salt Lake City.

Watkins, W. S., M. Bamshad, M. E. Dixon, B. Bhaskara Rao, J. M. Naidu, P. G. Reddy, B. V. R. Prasad, P. K. Das, P. C. Reddy, P. B. Gai, A. Bhanu, Y. S. Kusuma, J. K. Lum, P. Fischer, and L. B. Jorde
1999 Multiple Origins of the mtDNA 9-bp Deletion in Populations of South India. *American Journal of Physical Anthropology* 109(2):147–158.

Weir, Bruce S.
1996 Intraspecific Differentiation. In *Molecular Systematics,* edited by B. K. Mable, pp. 373–410. Sinauer Associates, Sunderland, Massachusetts.

Wrischnik, Lisa A., Russell G. Higuchi, Mark Stoneking, Henry A. Erlich, Norman Arnheim, and Allan C. Wilson
1987 Length Mutations in Human Mitochondrial DNA: Direct Sequencing of Enzymatically Amplified DNA. *Nucleic Acids Research* 15:529–542.