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Ancestral Puebloan mtDNA in Context of the Greater Southwest

Meradeth H. Snow

University of California, Davis Anthropology Department, Young Hall One Shields Avenue Davis CA 95616 mhsnow@ucdavis.edu (530) 848-1432

Kathy R. Durand

Eastern New Mexico University Kathy.Durand@enmu.edu

David Glenn Smith

University of California, Davis dgsmith@ucdavis.edu

Abstract

Ancient DNA (aDNA) was extracted from the human remains of seventy-three individuals from the Tommy and Mine Canyon sites (dated to PI-II and PIII, respectively), located on the B-Square Ranch in the Middle San Juan region of New Mexico. The mitochondrial DNA (mtDNA) haplogroups of forty-eight (65.7%) of these samples were identified, and their frequency distributions were compared with those of other prehistoric and modern populations from the Greater Southwest and Mexico. The haplogroup frequency distributions for the two sites were statistically significantly different from each other, with the Mine Canyon site exhibiting an unusually high frequency of haplogroup A for a Southwestern population, indicating the possible influence of migration or other evolutionary forces. However, both sites exhibited a relatively high frequency of haplogroup B, typical of Southwestern populations, suggesting continuity in the Southwest, as has been hypothesized by others (S. Carlyle 2003; Carlyle, et al. 2000; Kemp 2006; Malhi, et al. 2003; Smith, et al. 2000). The first hypervariable region of twenty-three individuals (31.5%) was also sequenced to confirm haplogroup assignments and compared with other sequences from the region. This comparison further strengthens the argument for population continuity in the Southwest without a detectable influence from Mesoamerica.

Keywords

Ancient DNA; mtDNA; Anasazi; migration

1. Introduction

The relationship between ancestral Pueblo people of the US Southwest and other populations in the region has long been the focus of archaeological attention. As Cameron (1995) notes, after a long period of neglect, migration has again moved to the forefront of our interpretations of population dynamics in the prehistoric Southwest (Clark 2001; Mills 2008; Reed 2008; Wilshusen and Van Dyke 2006; Windes 2007). While areas that experienced regional depopulation are easy to identify, determining the location to which people emigrated,

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Correspondence to: Meradeth H. Snow.

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particularly based on material culture, is less straightforward (Lathrap 1956). More recently, both modern and ancient DNA (aDNA) have been used to address migration hypotheses (Carlyle 2003; Carlyle, et al. 2000; Leblanc, et al. 2007; Malhi, et al. 2003).

Prehistoric and modern Native Americans belong to one of five mitochondrial DNA (mtDNA) haplogroups (defined by specific mutations that characterize shared ancestry), referred to as haplogroups A, B, C, D and X. The frequency distributions of these mtDNA haplogroups exhibit marked regional continuity in North America such that those of closely related or geographically contiguous populations are usually similar. For example, previous studies have reported a mtDNA haplogroup frequency distribution in prehistoric Pueblo remains that are characteristic of those observed in most modern populations in the Southwest, suggesting a population continuity for at least 1,000 years in that region (Carlyle, et al. 2000).

In the present study, we report the mtDNA haplogroups of 48 samples from two New Mexico sites and compare them with each other and with those of other prehistoric and extant populations in the Southwest and Mexico. Additionally, sufficient sequence from the first hypervariable region was collected from 23 (48%) of these 48 samples to confirm their haplogroup assignments and make limited comparisons with sequences from other extant Southwestern populations. For this purpose we adopt Beal's (1974) definition of the boundary between the "Greater Southwest" and Mesoamerica as the Mexican states of Nayarit and Jalisco and use the term "Southwest" to refer to the former region. These data provide further support for population continuity in the Southwest that has been previously hypothesized (S. Carlyle 2003; Carlyle, et al. 2000; Kemp 2006; Malhi, et al. 2003; Smith, et al. 2000), as well as possible regional and inter-regional migrations, such as those that have been suggested by Duff and Wilshusen (2000), Lekson and Cameron (1995), Varien, et al. (1996), Lekson (1999); as well as DiPeso (1974), LeBlanc (2002), Leblanc, et al. (2007), Matson (1991), and Turner and Turner (1999).

1.2 Modern and Ancient Mitochondrial DNA in the Southwestern United States

MtDNA, a small, circular, extra-nuclear DNA molecule housed in the cytoplasm of all human cells, has been the focus of human evolutionary studies because of its non-recombining (solely maternal) inheritance and high mutation rate. These features of mtDNA ensure that mtDNA haplotypes can be arranged in ancestor-descendant relationship to one another and that genetic differences can be identified among even relatively closely related individuals. It is particularly useful in studies of aDNA because its high copy number (in comparison to that of nuclear DNA) increases its probability of survival, allowing sufficient amounts of DNA to be extracted for analysis.

Following Schurr et al. (1990) and Torrini et al. (1993), most Native American samples belong to one of four common haplogroups: A, B, C, and D. Haplogroup X has also been identified in some Southwestern populations (such as the Jemez, Kiowa and Navaho), but was not tested in this study, as described below. Each of these haplogroups is defined by specific mutations that can be identified by restriction fragment length polymorphism (RFLP) analysis or direct sequencing of the base pairs found within the coding region of the mtDNA genome. Haplogroup A is defined by a Hae III site gain at np 663, haplogroup B by a 9 base pair deletion in region V, haplogroup C by a HindI site loss at 13259 and an Alu I site gain at 16262, haplogroup D by an Alu I site loss at np 5176, and haplogroup X by a Dde I site loss at nps 1715 and 10394. The definitive coding region mutations cited above are usually accompanied by typical mutations in the non-coding control region (CR) of mtDNA that can be used to confirm haplogroup assignments based solely on the coding region markers. The coding region site gain at np 663 that defines haplogroup A is usually accompanied by the CR mutations 16223T, 16290T, 16319A and 16362C. The nine-base pair deletion that defines haplogroup B is typically accompanied by the CR mutations 16183C, 16189C and 16217C. The HindI site

loss at 13259 and Alu I site gain at 13262 in the coding region of members of haplogroup C is usually accompanied by the CR mutations 16223T, 16298C, 16325C and 16327T (Malhi et al., 2003). The Dde I site loses at nps 1715 and 10394 that define haplogroup X are usually accompanied by the CR mutation 16278T. Haplogroup D lacks derived CR mutations accompanying its Alu I site loss at np 5176, although frequently mutations are seen at np 16223T and 16362C. The np 16223 is often regarded as a hypervariable loci, with a C→T transition found in four of the five haplogroups. Discussion of this locus is included here as it has been cited in previous literature (such as Tamm et al. 2007), although the site itself is not highly informative.

Due to the strong regional continuity exhibited by haplogroup frequency distributions in North America and Mesoamerica, comparative analysis of the frequencies of these haplogroups is a means of better understanding the relationships among populations in the two regions. MtDNA haplogroup frequencies in the Southwest are typified by high levels of haplogroup B, moderate frequencies of haplogroup C, and relatively low frequencies of haplogroups A, D, and X (Lorenz and Smith 1996). Such regional patterns result from common ancestry and shared population history and are maintained by mating patterns that reflect isolation by distance. The prehistoric Fremont to the north at the Great Salt Lakes (GSL) Wetlands (see Figure 1) also exhibit these typical Southwest haplogroup frequencies and, consequently, have been interpreted as a northern extension of Anasazi culture (Parr, et al. 1996). The haplogroup frequencies of Native American populations of Mesoamerica are equally distinctive, but haplogroup A is most common and haplogroup B is relatively rare (Kemp 2006; Malhi, et al. 2003; Smith, et al. 2000), suggesting different ancestry and/or population histories for the two geographic regions. Interestingly, the populations of northern Mexico, such as the Tarahumara, Cora and Huichol, exhibit intermediate frequencies of both haplogroups A and B, suggesting a cline incorporating the Southwest and Mesoamerica that might have resulted from gene flow between the two areas.

While previous reports on ancestral Pueblo populations in the Southwest have cited haplogroup frequencies in ancient populations similar to those found in extant populations in the Southwest (Carlyle, et al. 2000; Leblanc, et al. 2007), haplogroup frequencies alone do not provide unequivocal proof of genetic continuity over time or recent common ancestry between different populations. For example, Athapascan (Apachean) immigrants to the Southwest from the north are also distinguishable by their relatively high frequencies of haplogroup A, especially a version of haplogroup A that is restricted to circumpolar-populations (Malhi, et al. 2003), but this similarity reflects a very ancient, rather than a more recent, common ancestry between Athapascan and Mesoamerican populations. Similarly, the high frequency of haplogroup A in both Eskimo and Mesoamerican populations reflects very ancient rather than recent sharing of common ancestry because no highly derived mtDNA sequences (those due to very recent mutations) are shared between the two populations. By sequencing the HVSI of the mtDNA genome, close genetic relationships can be identified by the sharing of relatively rare recent mutations by two or more separate populations that are unlikely to have either independent or very ancient origins. Because most highly derived mutations in mtDNA are population-specific, additional mutations in the CR shared by some, but not all, members of any given haplogroup imply close genetic relationships or admixture among populations sharing them.

1.3 The Tommy and Mine Canyon Sites

The sites providing the aDNA for this study lie near the convergence of the Animas and La Plata Rivers with the middle San Juan River, in northwestern New Mexico (Durand, et al. 2010), as illustrated in Figure 1. This is a relatively lush area named the Middle San Juan or Totah region (McKenna and Toll 1992) located at the northern boundary of the San Juan Basin that would have provided ample farmland and wild game for its prehistoric inhabitants.

Communities in this region are thought to have been part of the Chaco culture (Lekson 1991; Vivian 1990), which was centered in the numerous great houses in Chaco Canyon. Although there is much debate about whether Chacoan communities were integrated into some type of formal political, economic, or religious system (Kantner and Mahoney 2000; Lekson 1999; Nelson 1995), the communities in this expansive region in the Four Corners area shared many aspects of material culture, such as a style of architecture (Cordell 1984), including the presence of large structures called great houses (Lekson 1984), similar types of ceramic wares (Cordell 1984; Katner 2004), and road features that spread out from the region's center in Chaco Canyon (Kincaid 1983; Nials et al. 1987; Vivian 1997a, 1997b). One of the roads heading north out of Chaco Canyon is thought to have ended near Salmon Ruins (Lekson 1999), one of the Middle San Juan Region's largest great houses that is 14 kilometers from the Tommy and Mine Canyon Sites.

The Tommy and Mine Canyon sites, the two source sites for our study, are part of the Point Community and are located on the B-Square Ranch, just south of Farmington, New Mexico (Wheelbarger 2008). There were five prehistoric communities on the B-Square Ranch, representing an occupational span ranging from the Pueblo I to Pueblo III periods (or approximately A.D. 800 to 1300). Four of the five communities contained a Chacoan great house, two of which were fairly large (the Fort Site with 50 to 100 rooms in the Animas Community, and Jaquez Site with 75 to 100 rooms in the Gallegos Community) and two of which were of unknown size due to severe erosion (the Point Site in the Point Community, and the Sterling Site in the Stewart Community). In addition, structures such as those representing the Tommy and Mine Canyon Sites, dot the regional landscape (Wheelbarger 2008).

Tommy Bolack, who owns and operates the ranch, carefully excavated numerous small test pits beginning in the 1960s and ending in 1989. Most of the excavation on the ranch occurred at the Tommy Site and the Mine Canyon Site. An examination of the ceramics from the burials at these sites suggests that the Tommy Site dates to the late Pueblo I/Pueblo II period (A.D. 800–1100), while the Mine Canyon Site was occupied in the Pueblo III period (A.D. 1100–1300). Although there may be a temporal overlap in their occupation in the early Pueblo III period, the majority of the analyzed ceramics from the Tommy Site date from the Pueblo I to late Pueblo II periods (73.4% of 1118 of 1522 sherds; Wheelbarger 2008:221–222). For the most part, the occupations of these two sites exhibit little overlap but were contiguous in time.

Over 100 human skeletons were excavated at the Tommy Site and 39 were recovered from the Mine Canyon Site. For our Tommy Site sample, 9% of the burials were intramural, 76% were in middens, and 15% were in other contexts (either between structures or adjacent to structure walls). The Mine Canyon Site sample had a higher percentage of intramural burials (41%), and burials in other contexts (22%), but fewer in middens (37%). These skeletal remains and their associated grave goods are currently housed on the B-Square Ranch. Although all amateur excavations on the ranch have been discontinued, San Juan College conducted field schools at the Tommy Site each summer from 1999 to 2006 (Wheelbarger 2008) that were funded by Bolack and San Juan College through a cooperative agreement defining the Totah Archaeological Project (TAP; Durand and Wheelbarger 2007). Because of the work of the San Juan College field school, more information is available for the Tommy Site than for the Mine Canyon Site (Wheelbarger 2008).

The Middle San Juan Region Osteological Project, funded by Bolack as part of the educational and research efforts of TAP, was conducted by one of the co-authors (KRD) and six of her graduate students. Their study included a portion of the genetic analysis presented here as well as thorough analyses of the skeletons using the standards established by Buikstra and Ubelaker (1994). These analyses included a paleodemographic study through time in the Point Community (Furhman 2007), a description of the paleopathology of each site (Adams 2007;

Cline 2007), a craniometric comparison of individuals across the northern Southwest (Greene 2007), a bone chemistry analysis (DeBoer and Tykot 2007), a faunal analysis of bones from the Tommy Site (Enright 2007) and a discrete dental trait analysis (K. Durand, et al. 2010; Durand and Wheelbarger 2007). These studies were designed to increase our understanding of changes in prehistoric health and diet in the region and the impact of the rise and fall of the Chaco system on small-scale regional populations (Durand and Wheelbarger 2007). In contrast to previous genetic studies of aDNA in the Southwest, like that reported by Carlyle (2003) that analyzed small samples from many sites that varied both in time and space, the remains at the Tommy and Mine Canyon sites represent relatively large samples of two discrete Southwestern populations that lived during adjacent time periods.

2. Materials and Methods

Two teeth were collected from 42 individual skulls sampled from Bolack's collection, one of which was sent to the Molecular Anthropology Laboratory (MAL) at UC Davis. Bone samples were also taken for bone-chemistry analysis from nine additional samples from the Mine Canyon site and 22 samples from the Tommy Site, and a small portion of each of these 31 samples was also sent to the MAL for aDNA analysis, for a total of 73 individual samples. These include a single sample (Kiva B) from remains found in a Kiva at the Tommy Site. DNA was extracted following the protocol described by Kemp et al. (2006) and Kemp and Smith (2005).

At least two independent DNA extractions were performed on each sample using the following procedures. A small piece of the root from each tooth (approximately 0.5 grams) was removed with a small saw, cleaned with 6% sodium hypochlorite (full strength household bleach; Kemp, et al. 2005), and soaked in 2mL of molecular grade 0.5M, pH 8.0, EDTA (Gibco) for at least 48 hours (an average time of seven days) to remove calcium. Small portions of each bone sample (0.2–1.3 grams) were broken into smaller fragments and treated in the same manner. Three milligrams of Proteinase K (Invitrogen, Fungal Proteinase K) were added to each sample and incubated at 65° overnight (an average time of 18 hours). A three-step phenol-chloroform extraction was carried out including two extractions with equal volumes (2mL) of phenol:chlorophorm:isoamyl alcohol (24:24:1; OmniPur), followed by one extraction with an equal volume of chlorophorm:isoamyl alcohol (24:1; Amresco). The DNA was then precipitated in a solution of isopropanol and 5M ammonium acetate overnight (for at least 12 hours) to remove PCR inhibitors. The DNA was then pelleted by centrifugation at 3100 rpm for at least 45 minutes, washed in 1mL of 80% purified ethanol, centrifuged for at least another 45 minutes, and allowed to air-dry for 15–20 minutes. The sample was purified using a Wizard PCR Preps Purification System (Promega) following the manufacturer's instructions, save for the final elution of the DNA using 100ul of ddH₂O.

These procedures took place in the aDNA facilities of the MAL, a positively pressurized clean room that is separated from the modern DNA extraction and PCR laboratories and to which access is strictly controlled. Uni-directional movement from the aDNA clean room and the PCR laboratory is maintained at all times, and precautions were taken to minimize risk of contamination at every step of the extraction process. Precautions included UV irradiating all supplies, performing duplicate independent extractions from, and amplification of, each sample, daily bleaching of all surfaces and tools within the room, use of protective clothing and running negative controls at each step. The mtDNA haplogroup and HVSI&II control region sequences of anyone having any contact with the samples and aDNA facilities in which the analyses were conducted were carefully recorded to ensure that the source of any laboratory contamination of the aDNA samples could be identified.

After extraction, regions containing the mutations diagnostic of haplogroups A, B, C, and D were amplified at least twice from each extraction in a different laboratory using the polymerase chain reaction (PCR) and primers, cycling conditions and methods cited in Kemp et al. (2006). After amplification of a PCR product of the expected size was confirmed by electrophoresis on 6% polyacrylimide gels, the DNA samples were digested with the HaeIII (for haplogroup A) and AluI (for haplogroup C and D) restriction enzymes (see Kemp et al., 2006 for further detail). The amplicons were incubated for at least 4 hours at 37°C and then run on a gel to check for digestion. Visual inspection of digestions for each sample revealed the presence or absence of the restriction sites characteristic of haplogroup A, C, or D. Haplogroup A was identified by a Hae III site gain at np 663, haplogroup C by an Alu I site gain at np 13261, and haplogroup D by Alu I site loss at np 5176. Haplogroup B, identified by a nine base pair deletion in region V of the mtDNA genome, was confirmed by its shorter fragment length after 6% polyacrylimide gel electrophoresis.

Fisher's exact tests were conducted using Genepop (Raymond and Rousset 1995) to compare haplogroup frequency distributions from relevant pairs of populations. Correspondence analysis (XLStat) was used to graphically represent the differences among the haplogroup frequency distributions of the different populations. The small sample sizes from both sites, and the Mine Canyon Site in particular, were taken into account throughout the process, as both of the statistical tests utilized are capable of handling small samples, especially Fisher's exact tests. Interpretation of the results was completed with the sample size in mind, particularly how it may influence the statistical significance of haplogroup similarities between sites.

Samples that could be assigned a haplogroup were sequenced to independently confirm their haplogroup assignments and provide more detailed genetic characterization of similarities among these samples and those from other populations in the Southwest and Mesoamerica. Sequencing was carried out by amplifying four overlapping 150-bp segments of the D-loop of the mtDNA control region from position 16045–16394, according to the Anderson reference sequence (Anderson, et al. 1981). Samples were sent to the UC Davis DBS sequencing facility for sequencing in both the forward and reverse directions and were also sequenced in the MAL using an ABI 3130 DNA sequencer. The primers and parameters used throughout this process are given in Kemp, et al. (2007). The completed sequences were analyzed and concatenated using the Sequencher and Mesquite (Maddison and Maddison 2007) computer programs and their mutations compared with those expected based on their haplogroup assignments to confirm those assignments. Samples assigned to haplogroup A were expected to exhibit most of their haplogroup's characteristic HVSI mutations, 16223T, 16290T, 16319A and 16362C, relative to the Cambridge Reference sequence. Those assigned to haplogroup B were expected to exhibit most of their haplogroup's characteristic mutations, 16183C, 16198C and 16217C. Those assigned to haplogroup C were expected to exhibit most of their haplogroup's characteristic mutations, 16223T, 16298C, 16325C and 16327T.

3. Results

3.1 Haplogroup Data

Haplogroup assignments could be made for 48 (65.7%) of the 73 samples sent to the MAL, more than doubling the number of samples from ancient Pueblo individuals whose haplogroups are known. The remaining 25 samples did not yield DNA the analysis of which could be replicated and were therefore not included in the analyses. In general, and somewhat unexpectedly, the Mine Canyon Site bone samples were more successfully amplified and haplogrouped than the tooth samples, possibly due to preservatives that may have been applied to the skulls provided by Bolack, which could have inhibited amplification. In no case did the first and second extractions or multiple amplifications of any of these 48 samples yield conflicting haplogroup assignments, and all samples exhibited the characteristic coding region

marker for one, but never more than one, of the four haplogroups. Both the Tommy and Mine Canyon Sites exhibited at least one instance each of haplogroups A, B, and C, as shown in Table 1. Haplogroup X had been previously reported in certain Southwest populations (Lorenz and Smith 1996) but was not tested in this study due to the inability to distinguish between valid mtDNA haplogroup results and contamination by the primary author who is a (non-Native American) member of haplogroup X, and because each of the 48 samples exhibited the mutation diagnostic of only one of the remaining four Native American mtDNA haplogroups.

The Mine Canyon Site exhibited a much higher frequency of haplogroup A than the Tommy Site (58.3% and 3%, respectively). The frequency of haplogroup B in the Tommy Site was high (69.4%), which is typical of populations in the Southwest (Lorenz and Smith 1996) but much higher than the frequency of this haplogroup at the Mine Canyon Site (33.3%). Both sites exhibited low frequencies of haplogroup C. The Tommy Site also exhibited a moderately low frequency of haplogroup D (13.9%), which has been reported in low frequency in other prehistoric Southwest populations (Leblanc, et al. 2007), including the Fremont (Parr, et al. 1996), but was absent at the Mine Canyon Site, as illustrated in Table 2.

The difference between the haplogroup frequency distributions of samples from the two ancient Pueblo sites was statistically significant ($p < 0.00001$, with the 0.05 level of probability corrected with Bonferroni's Correction to $\alpha = 0.0018$; Abidi 2007). Due to this difference, the two sites were separately compared to other populations, as is demonstrated in Table 3. While the small sample size of both sites could lead to the sites being erroneously regarded as distinct due to sampling error, as will be discussed later, the significant difference between the sites was regarded as sufficient to warrant separate comparisons of the two samples with other populations.

The haplogroup frequency distribution of the Tommy Site was not statistically significantly different from that of the ancient Anasazi samples reported by Carlyle ($p < 0.00344$, $\alpha = 0.0018$), and LeBlanc et al. (2007) ($p = 0.02282$, $\alpha = 0.0018$), which represent the only other large samples of prehistoric haplogroup data from the region. The Mine Canyon Site distribution was significantly different ($p < 0.00003$, $\alpha = 0.0018$) from that of both of these populations. The haplogroup frequency distributions of Carlyle's Western and Eastern subgroupings of Anasazi were not statistically significantly different from each other ($p = 0.10702$), and the frequency distribution of the Tommy Site did not differ from that of Carlyle's Western ($p = 0.00796$) or Eastern subgroup ($p = 0.11848$). The mtDNA frequency distribution of the Mine Canyon Site significantly differed from that of both the Eastern and Western subgroups of the Carlyle Anasazi sample ($p = 0.0004$ and $p = 0.00082$, respectively). The mtDNA haplogroup frequency distribution of the Tommy Site was statistically significantly different from those of all other populations included in Table 3 except the ancient Fremont site, while the mtDNA haplogroup frequency distribution of the Mine Canyon population differed from those of most southwestern U.S., but few of the ancient or extant Mexican, populations.

The correspondence analysis of relationships among the haplogroup frequency distributions of the populations summarized in Table 2 is illustrated in Figure 2. The first coordinate, F1, which predominantly differentiates between populations with high levels of haplogroups A (right half of plot) and B (left half of plot), explains 66.24% of the variance in haplogroup frequencies. Thus, Southwest populations, with typically high frequencies of haplogroup B, fall predominantly in the left side of the plot, while Mesoamerican populations, with high frequencies of haplogroup A, predominantly fall in the right side of the plot. The Tommy and Mine Canyon sites fall on opposite sides of the F1 coordinate, reflecting their different frequencies of haplogroups A and B. The Tommy Site forms a cluster with the Jemez, Fremont, and Zuni, and Carlyle's Anasazi data, reflecting these populations' high frequency of haplogroup B, typical of Southwestern populations. Consistent with the Fisher's Exact tests,

the Mine Canyon Site clusters most closely with Mesoamerican populations such as the Mixtec, Nahua and Maya, and with southern Athapaskan populations (Apache and Navaho) in the right half of the plot, due to these populations' high frequency of haplogroup A.

3.2 Haplotype Data

HVSI sequences were obtained for 23 of the 48 samples for which haplogroup assignments could be made. The forward and reverse sequences of each of these samples were consistent with each other and the mutations in the CR of all samples were consistent with their haplogroup assignments based on restriction analysis. The remaining samples did not amplify for the sequencing segments or yielded incomplete or poor data and were excluded from sequence comparisons. Most of the 23 sequences, given in Table 4, contain missing data that precludes the construction of informative haplotype networks and confounds comparisons of the sequences with those in other populations. The success rate of obtaining successful sequences is relatively low in relation to the percentage of samples that yielded haplogroup data primarily because only those sequences that were complete and had very small amounts of sequence ambiguity were used. While many of the other samples yielded sequence data that did not conflict with the haplogroup assignments, the data itself was deemed too poor to include in our analyses. Six of the 32 variable positions illustrated in the 23 sequences in Table 4 represent missing data and may not be variable. The remaining 26 variable positions include 22 transitions, two transversions (a G>C at 16188 and a T>A at 16263), and two insertions (a G at 16188 and a C at 16193). Irrespective of the identity of the missing data, these 23 sequences represent 17 unique haplotypes. Haplotype sharing was most common among haplotypes of haplogroups C and, though less so, haplogroup A, and least common among those of haplogroups B and D.

3.2.1 Haplogroup A—Haplogroup A is comprised of eight sequences from the Tommy and Mine Canyon Sites representing five unique haplotypes. Six of the eight sequences exhibited all four HVSI mutations characteristic of haplogroup A, 16223T, 16290T, 16319A, and 16362C, confirming their haplogroup assignments. MCS-R lacks the mutation at 16223, which could be due to a reverse mutation, and the nucleotide at 16362 in sample MCS-K could not be resolved (but is either a C or T, thus not precluding the presence of the fourth Haplogroup A-defining mutation).

One Mine Canyon sample, MCS-Z, represents the founding (or basal) haplogroup A haplotype that is common in many other populations, such as the Akimel-O'odham, Haulapai, Mixtec, and Zapotec. Since this haplotype is not derived, but rather represents very old common ancestry among these samples, its haplotypic similarity to other samples is uninformative of close genetic relationships. One sample from the Tommy Site (TS66) is one step removed (or more, if missing data mask additional mutations) from that sample with a unique transitional mutation (T>C) at 16311. This mutation occurs in members of haplogroup A in several other populations in both the Southwest and Mesoamerica but only as a multiple step mutation (Kemp et al., in press) and, therefore, it is unlikely to reflect a close relationship between any of them and the TS66 sequence.

MCS-D was the only haplogroup A sample that could be directly assigned to subhaplogroup A2 (defined by a C→T mutation at 16111), the most common sub-haplogroup of haplogroup A in the Americas, because the identity of the nucleic acid at this site could not be determined in the remaining samples of haplogroup A. However, HV2 sequence (data not shown) was successfully obtained from three haplogroup A samples from Mine Canyon (MCS-D, MCS-J and MCS-L) to clarify their subhaplogroup affiliation and all three exhibited at least one of the HV2 mutations diagnostic of subhaplogroup A2. In addition, only two of the eight samples assigned to haplogroup A could be confirmed not to be members of subhaplogroup A2.

Six Mine Canyon samples, representing two unique sequences, exhibited mutations at nps 16257 and 16263, both of which are shared with three Zuni samples and a single Tohono O'odham sample, all members of the A2 subclade of haplogroup A. Otherwise, these two mutations have been reported only in a single Chumash sample (Johnson and Lorenz, 2006). It is noteworthy that the only one of the eight samples that could be directly assigned to subhaplogroup A2 (MCS-D) also exhibited the 16257T and 16263A mutations. If the aforementioned Zuni, Tohono O'odham and Chumash sequences are indeed closely related to those six from Mine Canyon, the one of the Mine Canyon sequences with the 16257T and 16263A mutations that is also confirmed to lack the 16111T mutation (MCS-K) must represent a back mutation while the remainder whose nucleotide position at 16111 was undeterminable (MCS-F, MCS-J, MCS-L and MCS-R) must also be members of the A2 subclade (i.e., actually have the 16111T mutation as well), suggesting that that subclade of haplogroup A predominates in the Mine Canyon population. One of the six sequences in this clade, the single haplogroup A sequence lacking the characteristic C>T transition at 16223, was a more highly derived form of this sequence that also exhibited transitions at 16189 and 16221, absent from the otherwise matching sequences from the Zuni, Tohono O'odham and Chumash sequences cited above. The origin of the 16257T/16263A clade of A2 remains to be seen, but it is highly derived and provides evidence of cultural continuity between the Mine Canyon population and the current populations of the Southwest, but not those of Mesoamerica.

It is noteworthy that the mutations at 16233 and 16331 that characterize many haplogroup A samples in southern Athapascan populations are missing from the samples analyzed. Thus, the haplogroup A samples from Mine Canyon are unlikely to have derived from either Athapascan or Mesoamerican sources.

3.2.2 Haplogroup B—Each of the six haplogroup B sequences represents a different haplotype and all demonstrated mutations at either np 16183, 16189, or 16217. One of the six exhibited all three mutations while two exhibited only one (16183C or 16189C) of them. As 16183 lies in the poly-C region, the confirmation of haplogroup assignment of TSB8 based on presence of the 9-base pair deletion might be questioned, but the absence of diagnostic CR markers of other haplogroups provides no evidence that the TSB8 sequence is associated with a compound haplogroup.

The short sequence length and undeterminable nucleotide positions of some haplogroup B sequences did not reveal any clear relationships with those from the populations found in Table 2. However, four of the six samples (including samples from both the Mine Canyon Site and the Tommy Site) exhibited a C>T mutation at position 16111 that has been reported for the Zuni, Jemez Pueblo and other populations of the Southwest. This mutation defines a distinctive clade (B2a) in the Greater Southwest (including regions of northern Mexico) that has not been reported in any Mesoamerican population. Its haplotype network is star-like and its level of diversity is appropriate for a clade that expanded rapidly after the introduction of maize agriculture from Mexico (Malhi, et al. 2003; Kemp et al., in press). As positions beyond np 16394 were not sequenced in the present study, the presence of the 16483A mutation that almost always accompanies the 16111T mutation in this clade could not be used to confirm the membership of these sequences in subhaplogroup B2a. Thus, as for haplogroup A, the haplogroup B sequences appear to be indigenous to the Southwest and provide no evidence of connections between either the Mine Canyon Site or the Tommy Site to populations of Mesoamerica. However, one of two of the six sequences with the CR mutations 16183C and 16217C, but not 16189C, exhibited the mutation 16311C that has been reported from Mesoamerica, as well as the Zuni, Cora, Mixe, and Nahua (Kemp 2006). Interestingly, this sequence was obtained from the only sample recovered from a Kiva at the Tommy Site.

3.2.3. Haplogroup C—Five sequences were obtained from the haplogroup C samples, but all represent one of only two different haplotypes. Four of the five exhibited all four CR mutations characteristic of haplogroup C samples (16223T, 16298C, 16325C, 16327T) and the fifth exhibited three of the four, excepting only the 16327C mutation. None of the five sequences exhibits any additional (derived) mutations. As such, all five resemble this basal sequence of all haplogroup C haplotypes from many other populations (Cora, Huichol, Hualapai, Mixtec, Mixe, Nahua, Tohono O'odham, Akimel O'odham, Seri, Tarahumara, Zapotec, and Zuni) and are uninformative of closer genetic relationships.

3.2.4. Haplogroup D—There were only four haplogroup D sequences, all from the Mine Canyon Site, and all unique. One of the four sequences (TS-83) shared a G insertion at 16188 with the sequence of TS-26 and a C>T transition at 16223 with the sequence of TS-61. While, haplogroup D is relatively rare in the Southwest and Mexico, providing less opportunity for comparisons, and the four haplogroup D sequences described here are not closely related to those reported from other populations in either region, the addition of more samples in the future might reveal shared derived mutations between the individuals sequenced here, and those from other populations.

4. Discussion

Because all of the 48 samples could be assigned to haplogroups A, B, C or D, haplogroup X was assumed to be absent or to occur with such low frequency in both populations that it was not sampled, which is typical of most modern Native American populations of the Southwest. While compound haplogroups, those that contain the diagnostic coding region mutations associated with more than one of the five common mtDNA haplogroups in Native America, have been reported (Lorenz and Smith 1996), they are relatively rare and would not be expected to have been sampled in such a small sample as that studied here, even were they to exist in the two populations studied. The low frequency of haplogroup D in the Tommy Site and its absence at the Mine Canyon Site is not surprising, because this is typical of extant populations in the Southwest, with the exception of ancient and modern populations from the western Great Basin (Kaestle and Smith, 2001) and the Fremont site studied by Parr et al (1996). In fact, the unique failure for the haplogroup distributions of the Tommy Site and the ancient Fremont population to be statistically significantly different from each other supports Parr's conclusion that Fremont represents a northward extension of Anasazi culture. The frequency of haplogroup C at both sites is lower than that of many, but not all, extant Southwest populations. While the Tommy Site exhibited the high frequency of haplogroup B characteristic of extant populations in the Southwest, the Mine Canyon Site was atypical in having a higher frequency of A than of haplogroup B, like many extant Native American populations from Mesoamerica.

The close spatial proximity and slight overlap in the dates of occupation of the Tommy and Mine Canyon sites suggest that the residents of the Tommy Site constructed and gradually expanded into the Mine Canyon structures, eventually abandoning the Tommy Site. It was assumed that the emigrants from the Tommy Site moved down from the slopes of the Shannon Bluffs closer to the valley floor to establish the Mine Canyon Site near the beginning of the Pueblo III period. However, the results of the aDNA research presented here, as well as the discrete dental traits (Durand, et al. 2008), do not support such a simple population movement hypothesis. The differences in the haplogroup frequencies argue that something else may have occurred simultaneously to produce a more complex population history.

The evidence for genetic discontinuity reported here, in the form of markedly different haplogroup frequency distributions, suggests there may have been an influx of immigrants to the Point Community as the Mine Canyon Site was being established. Two migrations that may have affected the Middle San Juan region have been proposed: those associated with the

depopulations of Chaco Canyon to the south (around A.D. 1150; Lekson 1999) and the Mesa Verde region to the north (Duff and Wilshusen 2000; Lekson and Cameron 1995; Varien, et al. 1996; see Figure 1). Although the timing of the latter proposed migration is often proposed to have been around A.D. 1300, too late to have influenced the Mine Canyon population, several studies have suggested that it may have begun as early as A.D. 1150 (Duff and Wilshusen 2000; Varien 1999, although Varien et al. 2007 argue for a date in the late 1200s). Thus, there are two potential sources of immigrants to the Point Community, one from the south (Chaco Canyon) and another from the north (Mesa Verde region), who could have contributed to the Mine Canyon gene pool. A migration at the end of PII times that supplemented the more local emigrants from the Tommy Site to the Mine Canyon site could account for the difference in haplogroup A frequencies between these two samples as well as their sharing of high frequencies of haplogroup B. Further sampling within the Southwest, particularly that using contact DNA (Leblanc, et al. 2007), such as that extracted from quids, in order to comply with NAGPRA concerns, could help resolve whether either of these hypotheses provide viable interpretations of the prehistoric Southwest inhabitants. The discovery of unexpectedly high frequencies of haplogroup A, or haplotypes of any haplogroup identical or similar to those identified at the Mine Canyon Site, at Chaco Canyon and/or Mesa Verde would provide support for one or both hypotheses.

The relationship between Mexico and the Southwest has also been an issue of intense interest. Many theories regarding the entrance of foreign explorers, traders and warriors to the Southwest from Mesoamerica, where haplogroup A is far more common, have been proposed to explain the cultural and linguistic similarities between these two regions (for example: DiPeso 1974; LeBlanc 2002; Leblanc, et al. 2007; Matson 1991; Turner and Turner 1999). The recent identification of cacao use in cylinder jars from Pueblo Bonito in Chaco Canyon (Crown and Hurst 2009) provides further evidence of these connections. But whether such contact resulted in genetic admixture, population replacement, or simply cultural diffusion, has not been resolved. Recent studies of skeletal samples from northern Mexico found some sites with distinctive traits in comparison to their surrounding populations. In these studies, some northern Mexico samples provided evidence of gene flow with sites in the Southwest (Turner 1999), while other regional samples did not (Walker 2006). The intermediate frequencies of both haplogroups A and B in several populations of northern Mexico is consistent with such gene flow. The presence of high frequencies of haplogroup A at the Mine Canyon Site is interesting, because it has been argued that migrants would have brought haplogroup A with them from Mexico, where the haplogroup is found in high frequency (LeBlanc 2002; Leblanc, et al. 2007; Malhi, et al. 2003). Although it is tempting to attribute the high frequency of haplogroup A at the Mine Canyon Site to emigrants from Mexico, haplotype data obtained by sequencing do not support this. On the contrary, the haplotype data from the two sites reported here argue for population continuity within the Southwest based on the shared derived haplotypes of haplogroup A between the Mine Canyon Site and Zuni and Tohono O'odham individuals and the preponderance of the 16111T (B2a) clade of haplogroup B, both characteristics that are common in the Greater Southwest (taken to include northern Mexico) but absent from Mesoamerica. Thus, any population movement that might be responsible for the genetic difference between the Mine Canyon Site and the Tommy Site resulted from population movement within North America.

It is possible that gene flow from other populations outside Totah with higher frequencies of haplogroup A, such as Mesa Verde or Chaco Canyon (although these populations remain to be sampled), near the boundary between Pueblo II and Pueblo III significantly altered the haplogroup frequencies of the Mine Canyon Site but not those of the Tommy Site, which predated this immigration and may have been abandoned by this time. This hypothesis would be strengthened were mtDNA extracted from remains from Mesa Verde and/or Chaco Canyon and found to exhibit high frequencies of haplogroup A, including the highly derived 16111T/

16257T/16263A lineage. While previous research on ancestral Pueblo populations in the Southwest has reported haplogroup frequencies in ancient populations similar to those found in extant populations in the Southwest (Carlyle, et al. 2000; Leblanc, et al. 2007), haplogroup frequencies alone do not provide unequivocal proof of genetic continuity over time or recent common ancestry between different populations. For example, the high frequencies of haplogroup A in Eskimo, Athapaskan, and Mesoamerican populations reflect very ancient rather than recent sharing of common ancestry, because no highly derived mtDNA sequences (due to very recent mutations) are shared between any two of these populations. By sequencing the HVSI of the mtDNA genome, it was possible to establish close genetic relationships by identifying relatively rare recent mutations shared by two or more separate populations and geographic regions that are unlikely to have independent or only very ancient origins. Moreover, because correspondence analysis employed in our study gives less weight to the influence of less common haplogroups due to the chi-squared method it incorporates, caution should be exercised in the interpretation of the results illustrated in Figure 2.

Genetic drift, or the change in the frequencies of the haplogroups due to random events, could have affected the haplogroup frequencies of the sites as well. The occupants of the Tommy Site could have moved closer to the valley floor to occupy the Mine Canyon site, bringing with them an unrepresentative proportion of individuals with haplogroup A, perhaps because they were matrilineally related, creating a founder effect. Random events, such as inter-generational genetic drift or a population bottleneck, could also have skewed the haplogroup frequency distribution of the population of the Mine Canyon Site toward a high frequency of haplogroup A inhabitants. The small sample size available for this analysis could also lead to inadvertently sampling more individuals from a single matriline, leading to a higher frequency of haplogroup A. With its smaller sample size, the Mine Canyon Site could be heavily affected by such sampling errors. Obtaining a larger sample size from the excavated remains from both sites may allow for better understanding if sampling error is creating the distinct difference between the sites. While the statistically significant difference between the haplogroup frequency distributions of the Tommy and Mine Canyon sites could be due to cross-contamination, re-extraction did not support this, and the antiquity of the haplogroups in the Southwest, gene flow, or common ancestry between the two populations are more likely explanations. Even were the high frequency of haplogroup A in the Mine Canyon population due to sampling effects, no derived haplotypes are shared between the Tommy or Mine Canyon sites or populations outside the Southwest, and it is clear that the haplotypes from neither site are of Mesoamerican origin but rather represent ancient genetic relationships among populations in the Greater Southwest region.

Although the haplogroup frequency distributions differ between the two sites, they do share several related haplotypes. It is possible that gene flow from other populations outside Totah with higher frequencies of haplogroup A, such as Mesa Verde or Chaco Canyon (although these populations remain to be sampled), near the boundary between Pueblo II and Pueblo III significantly altered the haplogroup and haplotype frequencies of Mine Canyon but not those of the Tommy Site whose occupation predated this immigration. Further investigation of a larger sample from both sites and from other Pueblo sites will shed more light on this hypothesis.

5. Conclusions

The Tommy and Mine Canyon sites provide distinct snapshots of the genetic structure of prehistoric Pueblo populations. While the mtDNA of the population of the Tommy Site resembles that of other prehistoric (Anasazi) and contemporary populations in the Southwest (S. Carlyle 2003; Carlyle, et al. 2000), the Mine Canyon Site is very distinct. Although the sites may have been distantly related, or have experienced some gene-flow, something may

have occurred in the Point Community between PII and PIII times, such as a more distant migration. Migration from the Mesa Verde region in the north (Duff and Wilshusen 2000; Lekson and Cameron 1995; Varien, et al. 2007) or from Chaco Canyon in the south (Lekson 1999) may account for the differences. A migration from Mesoamerica is unlikely to account for the presence of haplogroup A at either site, nor is the presence of low frequencies of haplogroup B in Mesoamerica likely to have resulted from migrations from the Greater Southwest. However, further investigation of haplotype sequence data, including that from populations in Mexico known to have had contact with Pueblo populations in the Southwest, such as Casas Grandes in Chihuahua, Mexico, will provide a better evaluation of both hypotheses. Although the Mine Canyon Site did not conform to the general haplogroup pattern of the Southwest, the haplotypes it shares with modern populations within the Southwest, but not those in Mesoamerica, are indicative of a long period of population continuity, with minor migrations within the region and genetic drift explaining small discontinuities.

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Figure 1.

Locations of the Tommy and Mine Canyon sites in New Mexico, as well as other populations as noted in Table 2 (with the exclusion of Carlyle 2003, as many samples were taken from around the Southwest). The Mesa Verde and Chaco Canyon sites, mentioned in the text, are also noted.

Figure 2.

Correspondence analysis of haplogroup frequencies from Table 2. Red diamonds mark haplogroups. Blue dots mark Mexican populations, green squares mark Southwest populations, orange triangles mark Great Basin populations. See Table 2 for citations and other population information.

Table 1

Haplogroup Distribution of the Tommy and Mine Canyon Sites. Numbers in parentheses are the frequencies by site.

	Haplogroup A	Haplogroup B	Haplogroup C	Haplogroup D	Total
Tommy Site	1 (0.03)	25 (0.69)	5 (0.14)	5 (0.14)	36
Mine Canyon	7 (0.58)	4 (0.33)	1 (0.08)	0	12
Total	8 (0.16)	29 (0.60)	6 (0.13)	5 (0.10)	48

Table 2

Populations used for comparison in the correspondence analysis. Populations noted with a (**) are aDNA samples.

Population:	Haplogroup:						
	N	A	B	C	D	X	Citation:
Mine Canyon**	12	0.5833	0.3333	0.083	0	0	This study
Tommy Site**	36	0.0278	0.6944	0.139	0.1389	0	This study
Anasazi**	38	0.1071	0.7143	0.179	0	0	Carlyle 2003
Western Basketmaker II (skeletal + artifactual)**	23	0.13	0.783	0.043	0.043	0	Carlyle 2003 & LeBlanc et al., 2007
Akimel O'odham (Pima)	144	0.0479	0.4658	0.48	0.0068	0	Malhi et al., 2003 & Kemp 2006
Hualapai	76	0.0132	0.5	0.487	0	0	Kemp 2006
Tohono O'odham (Papago)	42	0.0714	0.5714	0.357	0	0	Kemp 2006 & Malhi et al., 2003
Aztecs**	23	0.6522	0.1304	0.044	0.174	0	Kemp et al., 2005
Cora	72	0.3056	0.5139	0.139	0.0417	0	Kemp 2006
Huichol	62	0.3065	0.5323	0.161	0	0	Kemp 2006
Maya	26	0.5385	0.2308	0.154	0.0769	0	Schurr et al., 1990 & Torroni et al., 1993
Maya Xcaret**	24	0.875	0.0417	0.083	0	0	Gonzales-Oliver et al., 2001
Mixe	52	0.3077	0.2885	0.289	0.1154	0	Kemp 2006
Mixtec	67	0.6716	0.209	0.075	0.0448	0	Kemp 2006
Nahua-Atocpan	50	0.38	0.4	0.18	0.04	0	Kemp 2006
Nahua-Cuet	46	0.6304	0.1957	0.152	0.0217	0	Kemp 2006
Tarahumara	73	0.3425	0.2877	0.315	0.0548	0	Kemp 2006
Zapotec	85	0.4235	0.2235	0.294	0.0588	0	Kemp 2006
Fremont **	32	0	0.75	0.13	0.06	0	Parr et al., 1996
Apache	29	0.62	0.17	0.14	0.07	0	Torrino et al., 1993
Navajo	58	0.52	0.41	0.03	0	0	Lorenz and Smith 1996
Jemez	107	0	0.8598	0.037	0.0093	0.1	Lorenz and Smith 1996, Kemp 2006
Zuni	70	0.1714	0.7429	0.086	0	0	Keastle and Smith 2001, Kemp 2006

Table 3

P-value comparisons between the Tommy Site, Mine Canyon Site, and the populations listed in Table 2. P-values calculated using Genepop (Raymond and Rousset 1995). Populations marked with a (**) are aDNA samples. As mentioned in the text, the inability to distinguish two populations statistically does not infer they are related, and caution should be employed in interpreting these results. Results marked with a (*) are statistically significant from one another at the 0.05 level, when corrected with Bonferroni's Correction ($\alpha=0.0018$).

	Tommy Site	Mine Canyon Site	Region
Carlyle's Anasazi**	0.00344	0.00003*	Prehistoric Southwest
Carlyle's Western Anasazi**	0.00796	0.00082*	Prehistoric Southwest
Carlyle's Eastern Anasazi**	0.11848	0.00004*	Prehistoric Southwest
Western Basketmaker II (skeletal + artifactual)**	0.02282	0.00021*	Prehistoric Southwest
Akimel O'odham (Pima)	<0.00001*	<0.00001*	Modern Southwest
Hualapai	<0.00001*	<0.00001*	Modern Southwest
Tohono O'odham (Papago)	0.00002*	<0.00001*	Modern Southwest
Aztecs**	<0.00001*	0.03024	Prehistoric Mexico
Cora	<0.00001*	0.07140	Modern Mexico
Huichol	<0.00001*	0.04606	Modern Mexico
Maya	<0.00001*	0.45149	Modern Mexico
Maya Xcaret**	<0.00001*	0.00328	Prehistoric Mexico
Mixe	<0.00001*	0.01579	Modern Mexico
Mixtec	<0.00001*	0.49419	Modern Mexico
Nahua-Atocpan	<0.00001*	0.32299	Modern Mexico
Nahua-Cuet	<0.00001*	0.46205	Modern Mexico
Tarahumara	<0.00001*	0.02898	Modern Mexico
Zapotec	<0.00001*	0.06074	Modern Mexico
Fremont **	0.34003	<0.00001*	Great Basin, USA
Apache	<0.00001*	0.32363	Modern Southwest
Navajo	<0.00001*	0.17160	Modern Southwest
Jemez	<0.00001*	<0.00001*	Modern Southwest
Zuni	<0.00001*	0.00001*	Modern Southwest

Variable positions within the HVSI of the Tommy and Mine Canyon Site samples. Hap refers to the haplogroup assignment of the sample. A "." denotes the sample is the same as the reference (Anderson 1981) sequence at the base pair. Y denotes an uncertain base call, where the sample was either a C or T at the location. N denotes an uncertain base call, where the sample was any one of the four basepairs at that location.

[illegible]

TS-61	D	.	.	.	.	C	.	N	N	G	.	.	.	.	T	.	Y	.	.	.	.	.	.	.	N	.	.	N
TS-90	D	.	.	.	.	R	.	C	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.
TS-26	D	.	.	.	.	.	.	N	N	N	G	.	.	.	.	Y	.	.	.	.	.	.	.	.	.	.	.	C
TS-83	D	.	.	.	.	.	.	N	N	N	G	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	N