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Additional analysis of mtDNA from the Tommy and Mine Canyon sites

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ABSTRACT

Ancient mitochondrial DNA analysis of samples from the Chaco outlier Tommy and Mine Canyon sites (dating to PII and PIII, respectively, and located near Farmington, New Mexico) originally revealed distinctly different haplogroup frequency distributions from one another. An additional twelve samples were added to those already published (Snow et al., 2010), bringing the total number of individuals with mitochondrial DNA data to 60. The additional samples diminished this difference but maintained the statistically significant distinction between the sites, pointing to a potential sampling bias at the Mine Canyon Site that might explain some, but not all, of the difference. The sites were compared with those from both modern and prehistoric populations in the desert Southwest and Mexico to better understand the regional context of the inhabitants, as well as inter-regional relationships, demonstrating that the Tommy Site fits well within the general Southwestern pattern, while the Mine Canyon Site stands out due to its higher than normal frequency of haplogroup A2. Thirteen burials from a kiva structure at the Mine Canyon Site are compared to the remainder of the excavated burials from the site in terms of mtDNA, burial position, pathology, and grave goods. The statistically significant differences of the kiva burials are discussed in terms of possible evidence of site abandonment, disease, slavery, and witchcraft.

1. Introduction

Prehistoric migration patterns can be difficult phenomena to document and, thus, may have been more common than we recognize today. Changes through time among residents of two sites in northwest New Mexico provide evidence of possible immigration of a non-local group into the region after 1100 CE. These sites, the Tommy and Mine Canyon Sites, are located along the San Juan River outside Farmington, New Mexico, on the B-Square ranch owned and operated by Tommy Bolack. The sites are part of the Point Community in the Middle San Juan region near the convergence of the San Juan, Animas, and La Plata rivers. Believed to be Chacoan outliers, four of the five known communities on the ranch have great houses, and many smaller communities, such as the sites focused on here, dot the landscape.

The original report on the genetic data from the site (Snow et al., 2010) demonstrated a distinct pattern that suggested significant differences between the two sites in terms of their haplogroup composition. The Tommy Site followed the more typical American Southwest pattern of high haplogroup B2, whereas the Mine Canyon Site maintained a high frequency of haplogroup A2. The original sample sizes from the sites were small, however, particularly from the Mine Canyon Site. For this reason, additional sampling was undertaken in order to clarify the genetic relationship, if any, between the two sites, and determine whether the Mine Canyon Site's distinct nature was due to sampling bias.

Samples were originally collected from remains excavated by Tommy Bolack to better understand the relationship between the two sites and investigate the genetic background of individuals living during the Pueblo I/II and III (800–1300 CE) time periods that the Tommy and Mine Canyon Sites, respectively, represent. Dates were obtained based on pottery at the sites, and currently no C14 dates have been successfully obtained. Ancient mitochondrial DNA (mtDNA) haplotypes and/or haplogroups of 48 individuals excavated from the two sites were reported previously (Snow et al., 2010). An additional 36 samples were collected from these sites (17 from the Mine Canyon Site and 19 from the Tommy Site), and with these additional samples that yielded analysable and replicated DNA, a total count of 40 samples are reported here for the Tommy Site and 20 from the Mine Canyon Site.

During the period in which the Mine Canyon Site was occupied, the Middle San Juan Region is thought to have experienced immigration from populations to the south in Chaco Canyon (Lekson, 1999) or from the Mesa Verde Region to the north (Duff and Wilshusen, 2000). Thus, one of the goals of this study was to test this hypothesis. With the Tommy and Mine Canyon Sites so close in space and time, would

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the aDNA of their skeletal assemblages reveal any evidence of immigrants coming into the region during the Pueblo III (Mine Canyon Site) period?

1.1. Mitochondrial DNA in the desert southwest

MtDNA is circular extra-nuclear genetic material found in the mitochondria of cells. It has been the focus of population genetics studies due to its high mutation rate and solely maternal inheritance that enable the identification of genetic differences within a population and the order in which these mutations occurred. By analysing mutations found within the ~16,500 base pairs, it is possible to identify maternal lineages, which are grouped into haplogroups and the haplotypes they comprise, each representing lineages in the branching human phylogenetic tree. Within the New World, different regions have mtDNA haplogroup frequency signatures composed of the five indigenous haplogroups most common to the Americas, known as A2, B2, C1, D1 and ×2, and their derived variants (Horai et al., 1993; Torroni and Wallace, 1995; Fagundes et al., 2008). The distribution of these different haplogroups is non-random and follows different frequency patterns in distinct geographic regions of the Americas.

By sequencing regions of the mtDNA molecule, especially the first hypervariable region (HVRI), it is possible to identify mutations that denote haplotypes that can then be compared among individuals within the same site and among different sites.

The mtDNA carried by the occupants of the Southwest follows a general pattern of a high frequency of haplogroup B2, low to moderate frequencies of haplogroups C1 and D1, and a low frequency or absence of haplogroups A2 and ×2 (Lorenz and Smith, 1996). This pattern is found in many of the populations in the Southwest, with the exception of Athapaskan populations (such as the Apache and Navajo) who carry a haplogroup frequency high in a derived circum-polar form of haplogroup A2.

There is also a distinction between the Southwest and populations further south in Mexico, where a higher frequency of haplogroup A2 and low to moderate frequency of haplogroup B2 is seen (Kemp, 2006; Malhi et al., 2003; Smith et al. 2000).

1.2. Previous research at the Tommy site and Mine Canyon site

It has been proposed that the residents of the Tommy Site moved onto the valley floor to occupy the Mine Canyon Site and, therefore, were closely related to one another. Other biological data collected on the same remains, such as the analysis of discrete dental traits (Durand et al., 2010), paleopathology (Adams, 2007; Cline, 2007), craniometrics (Greene, 2007) and bone-chemistry (DeBoer and Tykot, 2007), supported this hypothesis of a close genetic relationship between the residents of the two sites.

The haplogroups of 48 individuals from both the Tommy Site and Mine Canyon Site were previously reported (Snow et al., 2010) as reproduced in Table 1. When comparing the previous haplogroup frequencies with others in the region using Correspondence Analysis and Fisher's Exact tests, the Tommy Site clustered with other American Southwest sites due to its high frequency of haplogroup B2 and was statistically indistinguishable from the other ancient samples in the region (from Carlyle (2003) and LeBlanc et al. (2007)). In contrast, the haplogroup distribution from the Mine Canyon Site did not fit the general Southwestern pattern and was highly statistically significantly different ($p < 0.0001$) from all other sites from the Southwest, including the Tommy Site, due to its high frequency of non-Athapaskan haplogroup A2. Thus, the mtDNA evidence seemed to contradict this pattern of similarity between the sites.

Table 1
The results from the previous reporting of haplogroup frequencies at the Tommy Site and Mine Canyon Site (Snow et al., 2010), and those reported here.

Previous Haplogroup Results	Hap A	Hap B	Hap C	Hap D	N
Tommy Site	1 (0.03)	25 (0.69)	5 (0.14)	5 (0.14)	36
Mine Canyon Site	7 (0.58)	4 (0.33)	1 (0.08)	0	12
Including new samples					
Tommy Site	1 (0.03)	27 (0.68)	6 (0.15)	6 (0.15)	40
Mine Canyon Site	8 (0.4)	10 (0.5)	1 (0.05)	1 (0.05)	20
Total	9 (0.15)	37 (0.61)	7 (0.12)	7 (0.12)	60

The hypervariable region sequences from the Mine Canyon Site samples provided another intriguing result. Of the eight haplogroup A2 samples reported from the Mine Canyon Site, all had two derived mutations that set them apart from the haplogroup A2 samples at the same site and elsewhere, including at the Tommy Site, that did not carry either of the derived mutations. These mutations (at np16257T and np16263A) were also reported for several Zuni samples (Kemp et al., 2010), demonstrating that at some point the Zuni and Mine Canyon individuals shared a matriline.

A similar motif also has been found among the Chumash of California (Johnson and Lorenz, 2006; Kemp et al., 2010; Monroe et al., 2013). Trade between the two regions, as has been suggested by shell artifacts found in the Southwest that have California coastal origins (Cordell, 1997; King, 1990), may have led to recent gene-flow. Alternatively, if these mutations are homologous, they might be ancient ones that have become extinct in most other Native American populations.

2. Materials and methods

2.1. Additional sample collection

Because sampling effects may have affected the haplogroup distributions reported for the two sites, a larger sample of excavated burials was sought. More than one hundred burials have been excavated from the Tommy site and 39 burials have been recovered from the Mine Canyon Site. Samples from an additional 36 individuals (17 from the Mine Canyon Site and 19 from the Tommy Site) were obtained from the skeletal assemblages. The 36 samples were sent to the Molecular Anthropology Laboratory (MAL) at the University of California, Davis, for aDNA analysis. The findings from the resulting increased sample size are discussed below.

2.2. Ancient DNA

At least two independent DNA extractions were attempted on the new collection of bone and tooth samples. Protocols and procedures were nearly identical to those carried out on the previous samples (see Snow et al., 2010 for details). This included a phenol-chloroform extraction, followed by ethanol wash and spin column elution. Samples were amplified with PCR for haplogroup specific RFLP markers, and haplogroup assignments preliminarily designated through enzymatic cuts in the DNA and verified through acrylamide electrophoresis. This was followed by sequencing the HVRI region using five overlapping primer sequences.

Measures to avoid contamination with modern DNA were taken at every step. The ancient DNA facilities at the MAL are separate from those where modern DNA is handled, and movement is uni-directional between rooms. Laboratory materials and space were daily cleaned with a bleach solution and exposed to UV light as appropriate. Blank samples were run with each extraction to detect possible contamination. In no instance was modern DNA detected in any of the samples.

Comparison of the haplogroup frequencies with those of other populations from the Southwest and Mexico, listed in Table 2, were conducted using Fisher's Exact Tests (using Genepop, Raymond and Rousset, 1995), and the results reported in Table 3. The relationships among the populations were visualized using Correspondence Analysis (Nenadic and Greenacre, 2007), as illustrated in Fig. 1.

The first hypervariable segment (HVRI) of the mtDNA control region was sequenced and mutations were identified through comparison to