Supplemental Text

UNDERSTANDING TURKEY MANAGEMENT IN THE MIMBRES VALLEY OF SOUTHWESTERN NEW MEXICO USING ANCIENT MITOCHONDRIAL DNA AND STABLE ISOTOPES

Supplemental Text 1: Archaeological Site Summaries

Elk Ridge (LA 78963) is located by a spring at the confluence of a side canyon and the west fork of the Mimbres River on an alluvial fan surrounded by juniper, piñon, oak, and Ponderosa pine forest at an elevation of 6,360 ft (Berryman and Laumbach 2019). Elk Ridge is just beneath and adjacent to the earlier, Three Circle site (LA 53). Elk Ridge is one of the largest Classic period pueblo villages with an estimated 200 rooms, but it also has an earlier Late Pithouse period component with a great kiva with a flared ramp entrance like that found at Galaz (Creel and Anyon 2003). Neutron activation analysis by Creel and Speakman (2018) indicates that Elk Ridge was a major pottery production center. Currently, the Gila National Forest manages the northern portion of the site, while the Archaeological Conservancy owns the southern half of the site. Unfortunately, before the Archaeological Conservancy took ownership, pothunters searching for painted pottery vessels destroyed much of the southern Classic period room block in the 1980s. To mitigate damage from the pothunters, archaeologists with Human Systems Research, Inc. (HSR) excavated rooms and 17 human burials in the 1990s (Russell et al. 2006). More recently, the University of Nevada, Las Vegas (UNLV), in conjunction with the Gila National Forest excavated parts of the northern Classic period room block from 2015 to 2018 (Roth 2018; Roth and Creel 2017).

At this time, there are no absolute counts of how many turkey bones archaeologists have recovered at Elk Ridge since faunal analyses are ongoing from both the HSR and UNLV excavations. However, Morrison (2002) studied 17 turkeys from the HSR excavations, and there are at least seven additional turkeys from the UNLV excavations. Once faunal analyses are complete, there will likely be more than 24 turkeys from Elk Ridge. We sampled 10 turkeys from the HSR excavations, including birds that Morrison (2002) studied and seven turkeys from the more recent UNLV excavations.

The Lake Roberts site (LA 47821) is on the upper Mimbres River along the Sapillo drainage at an elevation of 6,060 ft. As part of a mitigation project, the Office of Contract Archeology at the University of New Mexico began excavating the site in 2011 (Seltzer-Rogers and Kurota 2021). The site primarily dates to the Classic period, but it also contains underlying features dating earlier in time. There is a masonry structure on the eastern end of the site, likely dating to the Classic period, but excavations at the western end identified four partially overlain Classic period pithouse structures. There was likely a mixed occupation within the site wherein portions may have been permanently inhabited, whereas others only seasonally. Geochemical analysis on the pottery indicates that people possibly consumed cacao drinks at the Lake Roberts site (Crown et al. 2015). Fourteen turkey bones were recovered during excavations, and we sampled three bones.

Mattocks (LA 676) is located on a terrace above the Mimbres River at an elevation of 5,900 ft. Nesbitt (1931) performed the first professional excavations at Mattocks, followed decades later by the Mimbres Foundation in the 1970s (Gilman and LeBlanc 2017; LeBlanc 1983:79-88). Gilman and LeBlanc (2017) summarize work at the Mattocks site, including discussion of the chronology, architecture, artifacts, and burials. Mattocks is one of the largest

and best-dated sites in the Mimbres Valley, with an estimated 180 rooms associated with eight pueblo room blocks (Anyon et al. 2017; Gilman and LeBlanc 2017). The occupation began around 550 and continued into the Classic period. Gilman (2006) argues that one family per room block lived there, and the site layout is different from other nearby Classic period pueblo villages. Gilman and LeBlanc (2017:187) state that turkey bones (*n* = 21 [number of individual specimens]) were well represented at Mattocks, yet they suggest turkeys were not kept on site. We sampled seven of those bones.

Old Town (LA 1113) is located along the lower Mimbres River an elevation of 5,000 ft. Along with the Galaz site, Old Town is one of the most important ritual and economic sites in the Mimbres Valley based on architecture, unusual burials, and exotic objects (Creel 2006a,b; Creel and Anyon 2003). Old Town was excavated in the early 1900s, and looters destroyed much of the site throughout the century. However, professional excavations by Texas A&M University began in 1989 and the University of Texas, Austin worked at the site during the 1990s and early 2000s (Creel 2006a). Excavations revealed people lived at Old Town for centuries, and several habitation areas reflect pithouse and pueblo occupations. Area A is a Classic period pueblo with a large pithouse component underneath. Area B is an isolated Late Pithouse period component. Area C is a later Black Mountain phase pueblo on top of a pithouse. Finally, area D is a large Classic period midden. In total, there could be as many as several hundred pithouses and potentially as many Classic period rooms.

Of the 627 bird bones recovered at Old Town, Creel et al. (2015:Table 2) report 47 identified turkey specimens (see also Cannon et al. 2014). Other than macaws, turkeys were the only deliberately interred bird at the site, including a juvenile turkey in the corner of room A2. We did not sample this bird because of concerns for destructive analyses. The two turkey bones

that we did sample were from general fill context from area A unit 32 feature A51(FS 4287 [distal humerus fragment of a juvenile]), and area A unit 22 room 16 (FS 4715 [distal tarsometatarsus fragment of a juvenile]).

Wheaton-Smith is located near the Gallinas Creek tributary of the middle Mimbres River at an elevation of 5,680 ft. This medium-sized pueblo was occupied during the Late Pithouse and Classic periods and was the main site in the Gallinas Canyon area. The single Classic period pueblo room block consists of approximately 25 rooms, and the Mimbres Foundation tested or excavated one-third of the rooms (LeBlanc 1977). Excavations revealed a large ceremonial room or kiva within the pueblo. Unfortunately, a more recent historic homestead occupation destroyed part of the Classic period pueblo. At least five turkey bones were recovered from Wheaton-Smith during the 1970s excavations. These include a complete right ulna, a distal left ulna, a complete left radius, and the two bones we analyzed.

See Supplemental Table 2 for additional provenience information on the 31 sampled turkeys.

Supplemental Text 2: mtDNA Analysis and Laboratory Methods

DNA extraction and analyses were performed by Ozga at the Arizona State University (ASU) for 24 turkeys from Elk Ridge, Lake Roberts, Mattocks, Wheaton-Smith, and Old Town. The 10 Elk Ridge turkeys Ozga analyzed derive from the HSR Elk Ridge excavations. At the University of Oxford (UO), Manin performed DNA extraction on seven Elk Ridge turkeys that derive from the more recent UNLV excavations (TU949, TU950, TU951, TU952, TU953, TU954, TU955). The samples were analyzed at two locations because Ozga moved to a new university and he could not complete the work in a high quality controlled laboratory in order to eliminate any potential contamination. Although the mtDNA work took place in two separate laboratories using different protocols (see below), the outcomes are reliable. The utmost care and caution were used by both analysts to prevent cross contamination between samples and any human contamination. Additionally, any potential ambiguous sequences were omitted for the analysis altogether, prevent any incorrect sequence data. The turkey genetic sequences have been deposited to GenBank (http://www.ncbi.nlm.nih.gov/genbank/) under accession numbers OP056179-OP056196.

All samples were extracted in a dedicated ancient DNA cleanroom using similar personal protective equipment (PPE). Throughout decontamination and extraction, full PPE gear, including Tyvek suits, hair nets, face masks, and googles, were worn to prevent the turkey bones from contamination by modern DNA. At ASU, the external surface of samples was decontaminated using a bleach wipe, and the outer layer of bone was removed using a sterile Dremel drill to further reduce contamination from environmental (soil) sources. Samples were crushed using a sterile hammer and underwent ultraviolet treatment for five minutes. A total of

50 to 250 mg of crushed bone was used for initial extraction (which included an extraction blank) using previously established protocols (Dabney et al. 2013). Concentrations were checked using Qubit dsDNA High Sensitivity Assay (Invitrogen), and values ranged from undetectable to 13.80 ng/ul, with no detectable DNA appearing in the extraction blank.

DNA was amplified in a modern DNA laboratory using four segments of the Hypervariable 1 Region from Kemp et al. (2017). Samples were Sanger sequenced using the University of Arizona Genome Center, and electropherogram outputs were manually confirmed and trimmed using Geneious 8 (ambiguities were left as 'N's). Results were then compared to Speller et al. (2010) and Kemp et al. (2017) to determine haplogroup assignment. Due to the presence of 'N' ambiguities and the lack of complete sequences at displacement loop (D-loop) nucleotide position (nps) 15554-16013 (a total of 460 base pairs [bp]), we were unable to establish finer scale resolution using the D-loop as documented in Speller et al. (2010). In other words, we could not identify turkeys as aHap1a, aHap1b, aHap1c, for example. Instead, all H1 and H2 haplogroups are labeled "aHap1?" and "aHap2?" similar to Kemp et al. (2017).

For the turkey samples extracted by Manin at UO, roughly 100 mg of each bone was sampled and decontaminated in a 1.5% sodium hypochlorite solution (NaOCl) and UV'ed for 10 minutes on each face. After being powdered, the samples were digested in a single step overnight, extracted using a silica-based protocol (Dabney et al. 2013), and built into double-stranded libraries using a Blunt End repair single-tube protocol (Carøe et al. 2018). Amplified libraries were then captured using a single reaction of a MitoBaits kit (based on the NC_010195.2 mitochondrial genome) and sequenced on a high throughout sequencing (HTS) Illumina HiSeq 4000 platform.

After demultiplexing, the paired-end reads were trimmed and collapsed using Adapter Removal v2.3.1 (Schubert et al. 2016). Next, collapsed and uncollapsed reads were aligned to the circularized mitochondrial turkey reference genome NC_010195.2 using the bwa back-track algorithm (Li and Durbin 2009). Finally, the reads were sorted, and the PCR duplicates were removed with samtools v1.10 (Danecek et al. 2021).

In order to match with the D-Loop region analyzed in the rest of the samples Ozga sequenced, the sequences were trimmed to the 15,554-16,719 bp region of the reference genome, and the coverage obtained for each sample was calculated on this same region (Supplemental Table S3 and Supplemental Figure S1).

In order to confirm the validity of the single nucleotide polymorphisms (SNPs) present in the HTS data and to take into account the limited coverage obtained for some samples (i.e., 52.75% to 92.37% of the bases covered with a 4.59 to 8.48-fold), the sequences were visually examined using the samtools view module. Transversions were considered valid when present in at least two independent reads. More care was taken to interpret transitions (C>T and A>G) as they are the natural by-products of the deamination occurring during DNA degradation, especially at the ends of the DNA fragments. Therefore, transitions were only considered valid when they were the exclusive allele present in at least three reads, including outside of the first/last five bp or when they were present in > 75% of the reads covering that base. No insertion or deletion was identified.

The haplogroup calling was performed based on a set of diagnostic SNPs from previously published D-Loop sequences from the region (Kemp et al. 2017; Speller et al. 2010). To be expected, no mutations characteristic of the H3 haplotype (ancient Mesoamerican and modern domestic breeds) were identified, and thus this category is not reported here (Supplemental Table

S4). However, all of the samples prepared at UO belong to the clade H1. The presence of certain mutations allows us to further push the identification for the three samples with the highest coverage (TU952, TU953, TU955), all corresponding to haplotype aHap1. Haplogroup calling was also performed on the 11 best preserved samples prepared at ASU using the same set of SNPs, confirming the presence of the H1 and H2 haplogroups.

We then compared these newly produced sequences to 619 published sequences of archaeological turkeys from the United States Southwest and Mesoamerica, modern and historical wild turkeys from the United States and Mexico, and worldwide domestic breeds (Supplemental Table S5; Canales Vergara et al. 2019; Luchini et al. 2001; Manin et al. 2018; Mock et al. 2001, 2002; Monteagudo et al. 2013; Padilla-Jacobo et al. 2018; Speller et al. 2010; Szalanski et al. 2000). Next, the sequences were trimmed to 411 bp, and considering the high rate of missing bases in our new sequences, a putative haplotype call was performed using DNAsp. Finally, relationships between haplotypes were displayed by a media-joining network using Network (v.10.2), removing the positions that were not covered in at least one individual (n = 14 haplotypes; Supplemental Figure S2) and taking into account the N's (n = 102 haplotypes; Supplemental Figure S3).

Supplemental Text 3: Stable Carbon and Nitrogen Isotope Analysis and Laboratory Methods

Stable carbon and nitrogen isotope analyses were performed by Krigbaum in the Bone Chemistry Laboratory at the University of Florida Department of Anthropology for 24 turkeys from Elk Ridge, Lake Roberts, Mattocks, Wheaton-Smith, and Old Town. The 10 Elk Ridge turkeys Krigbaum analyzed derive from the HSR Elk Ridge excavations. In addition, at the Archaeological Chemistry Laboratory at ASU, Schwartz and Knudson analyzed seven Elk Ridge turkeys that derive from the UNLV excavations. Results are provided in Supplemental Table S6.

Given recent research on inter-laboratory variability in carbon and nitrogen isotope analyses of bone samples, we focus on our interpretations on differences larger than the Minimum Meaningful Difference of 0.6‰ for $\delta^{13}C$ values and 0.9‰ for $\delta^{15}N$ values (Pestle et al. 2014). Also, both laboratories follow best practices for reporting stable carbon and nitrogen isotope measurements of ancient bone collagen (Guiry and Szpak 2021).

Collagen extraction and isotopic analyses for 24 turkeys from Elk Ridge, Lake Roberts, Mattocks, Wheaton-Smith, and Old Town were conducted at the Light Stable Isotope Mass Spectrometer Laboratory in the Department of Geological Sciences, University of Florida by Krigbaum. All faunal bone samples were mechanically cleaned and crushed with a ceramic mortar and pestle to retrieve fractions for analysis of bone collagen (0.25-0.5 mm), which were then placed into a centrifuge tube with 12 mL of 0.2 M hydrochloric acid (HCl). Collagen fractions were left to react with HCl for 24 h, centrifuged and decanted, and then refreshed with 0.2 M HCl every 24 h until the bone was fully demineralized. Samples were then rinsed to neutral pH in DI-H_2O, and humic substances were removed using 0.125M sodium hydroxide (NaOH) over a 20 h period. Ten mL of 1×10^{-3} M HCl were then added to the collagen in the

tubes, and the contents were transferred to 20 mL glass scintillation vials. Scintillation vials were placed in the oven, loosely capped, and left to sit 4–5 h at 95 °C. The solutions were returned to original test tubes, centrifuged, and the solutions were transferred back to the scintillation vials for evaporation in the oven at 65 °C. When ~2 mL remained in each scintillation vial, the solution was removed from the oven, capped, and placed in the freezer. Once frozen, the vial cap was loosened and placed in a freeze drier for 48 h and then weighed to determine collagen yield. Contents were placed in tin capsules and analyzed for $\delta^{13}C$ and $\delta^{15}N$ on a Carlo Erba Elemental Analyzer connected to a Delta V isotope ratio mass spectrometer.

Collagen extraction and isotopic analyses for seven Elk Ridge turkeys were performed by Schwartz and Knudson, following established laboratory methodologies. All faunal bone specimens were mechanically cleaned and reduced to bone fragments no larger than 3 cm in length. Bone fragments were weighed and demineralized over several weeks in glass vials containing 0.25M hydrochloric acid (HCl). Following the "chunk" method described in Sealy et al. (2014), the acid was changed every 48 hours until the bone was demineralized and became translucent and spongy. The samples were rinsed three times in distilled water, and then humic substances were removed using 0.125M sodium hydroxide over a 24-hour period. Collagen is then solubilized by adding 50 mL of a HCl solution with a pH of 3, covering the samples, and placing them in the oven at 90 °C for 24 hours. Excess HCl with a pH of 3 solution is dried off by leaving samples uncovered in the oven at 90 °C, and this process is repeated until each sample has fully solubilized. Samples are then re-hydrated with 3 mL of HCl solution with a pH of 3 and poured into 20 mL glass scintillation vials. Scintillation vials are then capped and placed in the freezer. Once frozen, the vial caps were removed, the vial was covered with a KimWipe secured

by a rubber band and then placed in a freeze drier. Samples were removed after 48 hours and weighed to determine collagen yield.

Collagen isotopic analyses were conducted at the Metals, Environmental, and Terrestrial Analytical Laboratory at ASU under the direction of Natayla Zolotova and Schwartz. Approximately one mg of sample collagen was weighed and placed in tin capsules. Samples were then analyzed for $\delta^{13}C$ and $\delta^{15}N$ on a Costech Elemental Analyzer coupled with a Thermo Electron DeltaPlus Advantage isotope ratio mass spectrometer following standardized procedures (Bond and Hobson 2012; Coplen 1994).

All carbon isotope ratios ($^{13}C/^{12}C$) are presented relative to the Vienna Pee Dee Belemnite (VPDB) international standard, and $^{15}N/^{14}N$ isotope ratios are presented relative to the atmospheric air (AIR) standard (van der Merwe 1982). $\delta^{13}C$ values are reported as a negative number because the Pee Dee Belemnite limestone has a higher $^{13}C/^{12}C$ ratio by comparison. At the Archaeological Chemistry Laboratory (ASU), analytical precision was determined through replicate sample analyses and was ± 0.02‰ for $\delta^{13}C$ and ± 0.002‰ for $\delta^{15}N$. Internal laboratory standards yielded mean $\delta^{13}C_{\text{glycine (VPDB)}} = -8.44 \pm 0.01‰$ (1σ, $n = 3$), mean $\delta^{13}C_{\text{tomato leaves (VPDB)}} = -27.02 \pm 0.06‰$ (1σ, $n = 6$), mean $\delta^{15}N_{\text{glycine (AIR)}} = +27.90 \pm 0.03‰$ (1σ, $n = 3$), and mean $\delta^{15}N_{\text{tomato leaves (AIR)}} = +4.11 \pm 0.03‰$ (1σ, $n = 5$). These values compare well with accepted lab standard values. Collagen sample integrity was examined through carbon to nitrogen (C:N) ratios and percent collagen yield (Ambrose 1990; De Niro 1985).

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