DOCUMENTING DOMESTICATION: MOLECULAR AND PALYNOLOGICAL ANALYSIS OF ANCIENT TURKEY COPROLITES FROM THE AMERICAN SOUTHWEST

By

BREANNE M. NOTT

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To the Faculty of Washington State University:

The members of the Committee appointed to examine the dissertation/thesis of BREANNE M. NOTT find it satisfactory and recommend that it be accepted.

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Brian M. Kemp, Ph.D., Chair

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John G. Jones, Ph.D.

_____

Jeremiah Busch, Ph.D.

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Abstract

By BreAnne M. Nott
Washington State University
May 2010

Chair: Brian M. Kemp

Although turkey (*Meleagris gallopavo*) is a favored food for many people worldwide, the domestication of the bird has received little academic attention. One study, which has attempted to rectify this lack of attention on turkey domestication, has identified a single domestic mitochondrial DNA (mtDNA) lineage in the American Southwest, one that represents an independent domestication from that which lead to the central Mexican domesticate (Speller et al. 2010). The current study builds on this previous research to document another domestic mitochondrial lineage from turkey coprolites at Turkey Pen Ruin, southeastern Utah.

Turkey Pen Ruin turkey coprolites exhibit two lineages previously identified as "aHap1" and "aHap2". Using a Fisher's exact test it was determined that mtDNA lineage frequencies at Turkey Pen Ruin deviate significantly from that exhibited by wild turkey populations in the region today (Merriam's turkey *Meleagris gallopavo merriami*). Pollen analysis of turkey coprolites also reveals no significant differences in turkey diet between the two lineages and further reveals pollen types of many cultivated and domestic crops used by prehistoric human inhabitants, indicating a close association between humans and turkeys at Turkey Pen Ruin. Based upon these lines of evidence, both aHap1 and aHap2 most likely represent domestic birds. This research therefore supports there historically being at least two domestic mitochondrial turkey lineages in the American Southwest.

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INTRODUCTION

Domestication

Domestication of animals has led to important food resources throughout the world. Bokonyi (1969) defined the domestication of an animal as the capture and taming of animals with specific behavioral characteristics, such as passive behavior and loyalty to family groups. Later definitions of animal domestication included characteristics of the domesticated animal itself and the cultural impacts of domestic species on human societies. Such changes can include alterations in diets and a reduction in population mobility of both human and animals (Hecker 1982). Ducos (1978), in emphasizing the cultural ramifications of domesticates on human societies, defined a domesticate as an animal integrated into a socioeconomic human group that while living, the domesticated animal is an object of ownership, inheritance, exchange, and trade. These cultural ramifications on human societies can be seen throughout the modern economies worldwide.

Ramifications to the domesticated animal, such as changes in morphology, also occur throughout the domestication process. Bokonyi (1969) described two phases in animal domestication, an animal keeping stage followed by an animal breeding stage. In the animal keeping stage, an animal is not selectively bred by humans for any specific trait and does not undergo significant morphological change. The animal is kept in an environment where food and care are not provided by humans, such as a large field or rock shelter. Over time the animal keeping stage is replaced by an animal breeding stage, during which the quantity and quality of food resources provided to the animal are controlled and care is provided. With controlled breeding of the domesticated animal, artificial selection is placed on the domestic animals, favoring the physical and/or behavior characteristics desired or subconsciously enforced by the

breeder. Outcomes of controlled breeding and artificial selection on a population of domestic animals include different sex and age compositions compared to wild populations, the disappearance of ancestral forms, and morphological changes over time (Bokonyi 1969).

Morphological changes, such as an increase in body mass over time of a species used for food, can be used to determine if humans had domesticated animals at archaeological sites. Nonetheless, the exact timing of when a species has become domestic and the purpose for initial domestication is often difficult to infer using historical methods. For example, in the case of turkey domestication some claim that turkeys were initially bred for feathers (Reed 1951, Rea 1980), while others argue for the consumption of the turkey early in prehistory (Hargrave 1939, Hargrave 1965, Breitburg 1985, 1993, Ferg 2007). Yet, predictions of a domesticated species can be made for what one would expect to see in the archaeological record. A domestic species used for ritual or other purposes, such as feather use, would be found with an articulated skeleton and without cut marks or burning (Hargrave 1939, Hargrave 1965, Senior and Pierce 1989, Munro 1994). While a species used for food should show signs of cut marks, fragmented bones, bones in middens, burning, and disarticulated specimens. Although domestication can be recognized through these zooarchaeological analyses of bones, domestication at sites without such bone specimens can often be difficult to be inferred and can add to the debate on the domestication of an animal species.

Basketmaker II: Origins, Agriculture and Turkey Husbandry

Despite an absence of zooarchaeological materials, cultural ramifications on human populations have been described as a way to infer domestication at archaeological sites. Hecker (1982) describes domesticates as having a strong cultural impact on human societies, such as a

heavier reliance on items produced by the domesticated animal. In the case studied here, during the Basketmaker II period in the Southwest [1000B.C.-A.D. 500 (Lipe 1999)], there are frequent indications of increased turkey feather use that could be indicative of a domesticated turkey population as described by Hecker (1982). Feathers were frequently used for costumes, feather bundles, prayer sticks, and other ritualistic activities (Wright 1914, Reed 1951, Schorger 1966, Schroeder 1968, Munro 2006, Bullock 2007). In Fresnal Cave, New Mexico, turkey feathers dated from 2500 B.C.-A.D. 1 were found in association with human habitations (McKusick 1980, 1986) and turkey feather blankets have also been recovered from the Grand Gulch, Utah (Morris 1939).

McKusick (1980, 1986) argues that by Basketmaker II times there was an increased use of turkey feathers; however very few bones have been observed in these sites thus making it difficult to interpret the true use of the turkey. Although scarce, turkey bones found in late Basketmaker II sites are associated with primitive pens and show evidence of healed bone breaks which Morris (1939) concluded to have been done by prehistoric people. Minimally, healed bone breaks are indicative of a close association between humans and turkeys. This could be due to a direct intervention such as humans mending turkey bones or from an absence of predators near human settlements. In either scenario, care of the animal was being provided and prehistoric people were in the animal breeding stage as defined by Bokonyi (1969).

Hecker (1982) further describes domesticated species as having a profound effect on human societies such as mobility and consumption patterns. For example, by incorporating maize, a domesticated species, a food resource was already available to feed captive animal populations, like the turkey. In the American Prehistoric Southwest, maize agriculture was prevalent in Basketmaker II (Kidder 1927).

Basketmaker II people were utilizing maize as a food resource (Morris and Burgh 1954, Berry 1982, Berry and Berry 1986, Matson 1991, 2002). Evidence of maize consumption by humans is found in two studies of stable isotopes that measured ^{13}C values (Matson and Chisholm 1991, Chisholm and Matson 1994). These studies revealed Basketmaker II human populations as having a high dependence on C4 plant species Such C4 plants include *Zea mays*, *Chenopodium* spp. and *Amaranthus* spp. and many others (Chisholm and Matson 1994). Although a C4 signature does not indicate only maize, these ^{13}C values were similar to later Puebloan groups who were heavily dependent on maize agriculture (Matson and Chisholm 1991). By utilizing wild plant species and cultivated crops, such as maize, Basketmaker II groups would have been able to feed themselves and an animal domestic like the turkey. In other words, a stable food resource, maize, was already a part of Basketmaker II culture and could have been utilized to support domestic turkey populations.

Several origins for initial turkey domestication in the American Southwest have been proposed. First, the domestication of the turkey is hypothesized to parallel the domestication of maize agriculture. Maize agriculture began in Mesoamerica and diffused into the American Southwest. Thus one possibility involves the arrival of domesticated turkeys into the American Southwest from Mexico following the adoption of maize agriculture (McKusick 1980). Others argue that the origin of turkey domestication was in the American Southwest from already present wild turkey populations, independent of those birds domesticated in central Mexico (Schorger 1966, Breitburg 1988). All of these studies, however, are limited by a complete absence of turkey specimens from the early Holocene making a zooarchaeological analysis of the origin of turkey domestication impossible (Munro 2006, McKusick 2007). Moreover, early North American turkey zooarchaeological samples appear concurrently in the archaeological

record, displaying no chronological or morphological pattern that could be used to infer domestication throughout the region, further obscuring an origin for the bird's domestication (Reed 1951, Schorger 1966, Breitburg 1988, Munro 2006).

Due to the absence of morphological patterns, other materials and/or methods of inferring domestication are needed. Recently, Mock et al. (2002) and Speller et al. (2010) have shed new light on the origin of turkey domestication by using molecular tools to conclude that turkeys were domesticated at least twice, once in the Southwest and once in Mesoamerica. Molecular studies, like those of Mock et al. (2002) and Speller et al. (2010) have been able to address domestication without the need for zooarchaeological material.

Domestication: Molecular and Palynological Approaches

Recently, molecular data using variable sites in the mitochondrial DNA (mtDNA) or chloroplast DNA (cpDNA) genomes, are being used to address domestication (Paabo et al. 2004, Zeder et al. 2006). For example, using modern and prehistoric cow DNA from the Middle East and Europe it was determined that ancient European cows did not contribute to modern domestic cow populations, giving credence to the domestication of the cow in the Middle East (Bailey et al. 1996).

Mock et al. (2002) used molecular analyses to characterize genetic differences both among and between 5 different subspecies of turkey. Using amplified fragment length polymorphisms, microsatellite analysis, and mtDNA, Mock et al. (2002) found that genetic differentiation of ancient domestic turkey populations (*Meleagris gallopavo merriami*) in the American Southwest implied a long separation from other subspecies. Further no genetic support was found for the origin of *M.g. merriami* from an Eastern or Mesoamerican turkey

population, suggesting a probable origin for turkey domestication somewhere in the American Southwest.

Speller et al. (2010), using ancient DNA (aDNA), have analyzed ancient mt(DNA) from 149 turkey bones and 29 turkey coprolites from 38 archaeological sites (200B.C.-A.D. 1800). This study indicated at least two centers of domestication for the turkey with a probable domestic turkey lineage, aHap1 that developed in one of the domestication centers (Speller et al. 2010). Speller et al. (2010) found that a single mitochondrial lineage (aHap1) is conserved in the American Southwest for approximately 1,500 years suggesting that the aHap1 was a domestic lineage. A second lineage, aHap2, was concluded to be a wild turkey lineage. Lineage aHap1 was predominant throughout the prehistory of the American Southwest and displayed signs of a genetic bottleneck and controlled breeding, indicative of domestication (Speller et al. 2010). By focusing solely on molecular data, Speller et al. (2010) overlooked other methods to determine domestic turkey lineages such as palynological analysis from the same specimens. This research attempts to assess previous conclusions by Speller et al. (2010) and determine if aHap2 is domestic turkey lineage using additional turkey coprolites and pollen analysis.

Palynological analysis, or pollen analysis, is another well developed tool to reconstruct domestication. Domestication often involves a change in dietary consumption and pollen analysis can be used to reconstruct diet. Turkeys feed by biting plant material and catching insects on the ground and would incorporate pollen directly from plant material they were eating (Schorger 1966). Pollen would further be incorporated into the feces of turkey coprolites by other direct and indirect methods.

Direct ingestion of pollen, either through the ingestion of plant material with adhering pollen grains, consumption of other feces, ingestion of soil or the consumption of pollen

contaminated water would result in pollen contained within the turkey coprolites (Bryant 1974, Reinhard et al. 2006). Some plant material, such as most mature fruits should not have significant amounts of pollen, due to environmental factors such as wind and rain that wash pollen off of the surface (Bryant 1974). Nonetheless, other plant materials such as flowers, nectar, and inflorescences contain large amounts of pollen that are easily consumed and would largely contribute to pollen observed in a palynological analysis. Likewise, direct consumption of other animal feces such as human coprolites would also serve to incorporate many pollen grains into the turkey coprolites analyzed in this study (Bryant 1974, Reinhard et al. 2006).

Pollen found within the soil may also be included within turkey coprolites (Bryant 1974, Reinhard et al. 2006). Turkey feeding involves foraging for insects and plant material frequently found on the soil surface. Often when feeding, turkeys consume stones, some of which end up in the digestive track as gizzard stones (Schorger 1966). Likewise, soil containing ambient pollen can also be incorporated into the turkey coprolites via this method. Turkey coprolites analyzed in this study contained pieces of insect chitin indicating a high probability of insect ingestion and the possibility of ambient pollen consumption.

Water with environmental pollen is another method of pollen introduction into feces. During peak pollen producing seasons, many pollen grains can become incorporated into water and when consumed, will contribute pollen into turkey feces. The direct ingestion of pollen by any of the aforementioned ways likely results in large concentrations of pollen and can include rare pollen types, such as zoophilous pollen (animal dispersed) that are produced in low quantities by the plant, into the feces of the animal.

Indirect ingestion of pollen from the air would also contribute to pollen found within each turkey coprolite. Wind-dispersed pollen, anemophilous pollen, is often produced in very high

quantities and can be carried by air currents a large distance (Bryant 1974, Reinhard et al. 2006). Wind-dispersed grains are typically light and can easily mix into air currents and can become part of the pollen spectra of coprolites when inhaled (Reinhard et al. 2006). Anemophilous grains in the soil may also become incorporated into air currents during soil mixing and inhaled (Bryant 1974, Reinhard et al. 2006). Further, after deposition of the coprolite, the surrounding environmental pollen in the air and soil could also contaminate and be included within the coprolite.

Although environmental pollen can contribute to the pollen taxa found within the diet of ancient organisms, pollen grains found within a coprolite analysis would reveal dietary pollen. Direct or indirect ingestion of environmental pollen contaminants would result in low quantities of environmental pollen. Pollen directly ingested from plant material would be present in large quantities throughout the coprolite and would mask low concentrations of environmental pollen. Further by analyzing soil pollen using palynological analysis, environmental pollen can be identified and dietary pollen can further be elucidated.

Prehistoric human and animal diets can be reconstructed by determining pollen types and identifying plant fragments present in prehistoric feces. Domestication of an animal species often results in a restriction of dietary diversity and would result in a restriction of pollen diversity. In other words, after an animal breeding stage is developed, the diversity of an animal's diet decreases often paralleling human diet. In this study, one focus is to catalogue and describe any parallels found between turkey and human diet based upon pollen types.

Martin and Sharrock (1964) conducted palynological analysis of preserved human Basketmaker II feces in the American Southwest. Maize pollen was present among these feces along with other pollen such as *Pinus, Quercus, Populus,* and *Juniperus* supporting a heavy

reliance of these species as food resources and supporting the cultivation of maize by Basketmaker II people (Martin and Sharrock 1964, Aasen 1984). Not only has diet of ancient humans been reconstructed via a palynological coprolite analysis, but the diets of other species such as ancient dogs and hyenas as well (Taylor and Scott 1983, Scott 1987) This study conducts both a molecular and palynological analysis of prehistoric turkeys to infer turkey domestication during Basketmaker II.

Turkey Pen Ruin

Turkey Pen Ruin, in Grand Gulch, Utah, is a Basketmaker II site which provides an ideal location to study turkey domestication. Previous studies have suggested that the American Southwest could be a potential origin of turkey domestication and therefore the study of turkey domestication during Basketmaker II in this region could provide important insight on the domestication of the turkey. Preservation of archaeological materials at Turkey Pen Ruin is also excellent due to low bacterial degradation common among most American Southwestern sites. The Grand Gulch was first surveyed in 1878 by the Hayden Survey and was classified as a part of the Cedar Mesa (Aasen 1984). In the 1890s this area was investigated by ranchers McLoyd, Graham, and the Wetherhills (Nordenskiold 1893). Their excavations uncovered materials such as hide bags, ceramics, tools, sandals, and numerous human and animal coprolites (Nordenskiold 1893).

In the 1970s, Matson and Lipe (1975, 1978) conducted the Cedar Mesa Project which entailed multiple surveys and excavations including Turkey Pen Ruin. Further in 1972, R.G. Matson and an excavation team dug into the archaeological midden at Turkey Pen Ruin (Matson and Lipe 1975, 1978). The square column excavated by this team was 1.4 m deep consisting of

hundreds of human and turkey coprolites, some of which were later used in this analysis (Aasen 1984).

Although zooarchaeological analyses are common in studies of domestication, turkey bone was not abundant in the midden excavated at Turkey Pen Ruin. Pollen and macrofossils, remains of plant materials, however can be found in large quantities throughout Cedar Mesa, including Turkey Pen Ruin. Over 20 different pollen types have been observed in varying concentrations in different environments all found within Cedar Mesa (Wodehouse 1935, Martin 1963, Martin and Byers 1965, West 1978). Examples of some of these species include Poaceae (Grass family), Cheno-Ams (both *Chenopodium* and *Amaranthus)*, *Sarcobatus* (Greasewood), *Eriogonum* (Buckwheat), *Sphaeralcea* (Globe Mallow), *Cleome* (beeweed), *Pinus* spp. (Pine), *Juniperus* (Juniper), *Salix* (Willow), *Fraxinus* (Ash), *Quercus* (Oak), Asteraceae high and low spines, and *Celtis* (Hackberry).

Since the discovery of Turkey Pen Ruin, many studies have analyzed plant material from this site. Lepofsky (1986) conducted a preliminary flotation sample from Turkey Pen Ruin and identified willow catkins, pinyon nuts, maize, *Chenopodium* spp. and *Amaranthus* spp. seeds, Indian rice grass, prickly pear seeds, sunflower seeds, and *Cucurbita* spp. seeds in samples from Turkey Pen Ruin. Most floral species found by Lepofsky (1986) represent a late summer/fall occupation for Turkey Pen Ruin with two species representing an occupation earlier in the year. In addition, Aasen (1984) identified that inhabitants of this site were agriculturalists with domesticated and semi-cultivated crops.

Despite previous floral data analyzed from Turkey Pen Ruin, little has been analyzed in regards to turkey domestication. One preliminary palynological study of turkey coprolites from Turkey Pen Ruin suggested two categories of coprolites representing wild and domesticated

turkeys, respectively (Arakawa et al. 2001). These categories were based upon relative maize concentrations found within turkey coprolites themselves, but did not provide an in-depth description of the prehistoric turkey diets.

Similar to most Basketmaker II sites, turkey bone is virtually absent from the midden at Turkey Pen Ruin (Powers 1984) and thus other analyses shall be used in this current study to conclude turkey domestication. This current study analyzed both previously identified molecular lineages present in turkey coprolites from Speller et al. (2010) and additional lineages determined here in conjunction with a palynological analysis of prehistoric turkey coprolites to analyze for dietary indications of turkey domestication.

Hypotheses and Expectations

This study represents a new investigation of turkey domestication, combining both molecular tools with a palynological analysis to determine turkey domestication in southeastern Utah. In order to reach a conclusion about turkey domestication at Turkey Pen Ruin, three predictions were addressed.

First, using lineage data collected via molecular method a Fisher's exact test explored the probability that wild birds (i.e. *M. g. merriami*) were randomly depositing feces at the site. Turkey populations exhibiting an aHap1 lineage have been previously identified as being a domestic species, while aHap2 lineage are concluded to be from wild birds (Speller et al. 2010). Thus, if Turkey Pen Ruin inhabitants had domesticated turkey the maternal lineage, aHap1 should be present at the site. Further if birds carrying aHap2 or any other "wild" lineage randomly depositing feces at the site, a Fisher's exact test should deduce no significant

differences in the frequency of each lineage found at Turkey Pen Ruin compared to the lineage frequencies found in wild populations.

Secondly, an attempt was made to reconstruct ancient turkey sex ratios represented at Turkey Pen Ruin. Estimates of modern wild turkey populations estimate an approximately equal ratio of male to female turkeys (Collier et al. 2007). One study of *Meleagris gallopavo intermedia* found a higher abundance of males in wild turkey populations with a brood sex ratio of 56% male turkeys (Collier et al. 2007). If turkeys are domesticated at Turkey Pen Ruin, sex ratios of domestic populations would deviate significantly from wild populations with a higher female ratio in domesticated populations than recorded for wild populations. One male turkey can fertilize the clutches of multiple females, thus only a limited number of males are required and a higher female:male ratio is expected in domesticated populations.

Thirdly, similarities of diet in a previously identified domestic lineage, aHap1 and a previously identified "wild" lineage, aHap2, are discussed. One indication of domestication includes the change in dietary consumption by the species being domesticated. Domestication of the turkey would involve changes to turkey diet including a restriction in the number of consumable species and a higher emphasis on cultivated/domesticated crops like maize. If turkeys were domesticated at Turkey Pen Ruin, then a palynological analysis will reveal pollen types of cultivated or domesticated plant species by Basketmaker II people. Further, if the reported "wild" lineage aHap2 is not domesticated, then a pollen analysis should reveal significant differences in pollen composition when compared to the reported domestic lineage aHap1.

Lastly, the diet of turkey coprolites examined in this study was compared to human coprolites analyzed by Aasen (1984). Maize, one of the first domesticates in the American

Southwest, was well established during Basketmaker II times, thus allowing the subsequent use and domestication of other plant and animal species, like the turkey. A domestic species in the animal breeding stage requires human care and feeding. Therefore, if turkeys are domesticated at Turkey Pen Ruin, similarities between turkey diet and human diet should exist.

Ultimately, using molecular and palynological analyses, the goal of this study was to document domestic turkey lineages, specifically the domestication of lineage aHap2, among turkey coprolites at Turkey Pen Ruin, southeastern Utah.

METHODS

Sample Acquisition

Excavations at Turkey Pen Ruin (42SA3714) by R.G. Matson in 1972 revealed an undisturbed midden next to a masonry-lined pithouse. Before removing any sediment from the midden column, a profile of strata was produced of the exposed midden (Figure 1). Sediments were then removed by strata and placed in large bags and transported to the Washington State University Museum of Anthropology for analysis (Aasen 1984). In 1973, some of the bags and strata were screened for a preliminary study of the midden assemblage. Half of the contents of each stratum (A-3, A-5, B-1, B-3, C-1, C-2a, C-2c, C-5, D-2, and D-3) were screened through 120 µ, 180 µ, 250 µ, 500 µ, 850 µ, and 1.70 mm soil sieve screens. Remnants of each screening was stored in bags and kept for further analysis (as cited by Aasen 1984, Powers 1984).

Coprolites from Turkey Pen Ruin midden were collected from the WSU Museum of Anthropology warehouse on 4/21/2009 by William D. Lipe with later collections by Brian M. Kemp (Appendix 1). This study uses a subset of those turkey coprolites collected by W.D. Lipe and B.M. Kemp. Bone fragments (Appendix 2) and soil samples (Appendix 3) were collected from October 2nd-6th, 2009. Provenience data given in each Appendix (1-3) are copied from the storage records of the WSU Museum of Anthropology.

Contents of each bag (Appendix 4) were sorted through and a random sample of turkey coprolites was collected. All bone fragments encountered in these bags were collected. During collection, museum storage bags were visually inspected for the preferred material while personnel were properly attired to control for DNA contamination following standard procedures at WSU (*See DNA Extraction)*. Each bag was sorted through independently of each other to minimize contamination between bags. All collected turkey and mammal bone were then stored

in the Ancient DNA Laboratory. Each selected turkey coprolite and bone was subsequently prepared for DNA extraction and the coprolites further prepared pollen analysis.

DNA Extraction

DNA extraction and analysis were performed, as described below, following Kemp et al. (2007) and Speller et al. (2010). A variety of procedures were conducted in order to reduce and eliminate contamination during DNA extraction and pollen analysis. During retrieval of samples from storage at WSU Museum, all personnel were required to wear clean lab coats, gloves, hair nets, and face masks to reduce modern contamination. Portions of each coprolite were used for the extraction of aDNA and each coprolite was only exposed for a short time in the ancient DNA clean room in order to reduce contamination. PCR amplification and post-PCR procedures were conducted in a separate building to further reduce the likelihood of modern contamination.

A portion of each coprolite (0.05-0.81g) or bone (0.01-0.17g) sample was removed from the whole for DNA extraction. The remaining coprolite material was transferred to the paleoenvironmental laboratory at Washington State University to further analysis. Bone samples were submerged in 6% sodium hypochlorite (full concentration Clorox bleach) for 15 min to remove all outside modern DNA contaminants (Kemp and Smith 2005). The bleach was removed by rinsing the samples with DNA free water (ddH_20). Coprolites were not subjected to a bleach treatment. All samples, bone and coprolite, were transferred to 15 mL conical tubes and each sample was then immersed in 3 mL of 0.5 M molecular grade EDTA (pH 8.0) for more than 48 hours. An extraction control (a conical tube with only reagents and without a sample) was included with every extraction in order to test for possible contaminating DNA. To each sample,

3 mg of proteinase K was added and the samples incubated at 60-65°C for 3 hours in order to destroy all DNA-degrading nucleases and breakdown any other proteins. DNA was further extracted by adding an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) to the EDTA and centrifuged at 3,100 rpm for 5 minutes. The aqueous phase was removed and further extracted using phenol:chloroform:isoamyl as described above. After centrifugation the aqueous phase was extracted using chloroform:isoamyl alcohol (24:1) which was mixed and centrifuged at 3,100 rpm for 3 minutes. The supernatant phase from this reaction was added to a new conical tube with one half the volume of 5 M ammonium acetate. An equal amount of isopropanol was added to the tube and stored overnight for DNA precipitation.

After DNA precipitation in ammonium acetate and isopropanol, each sample was centrifuged for 30 min at 3100 rpm to pellet the DNA. After centrifugation, the supernatant was discarded and samples were air dried for 15 min. The pelleted DNA was then washed in 1 mL of 80% ethanol and vortexed for 30 seconds. All samples were then centrifuged for another 30 minutes at 3100rpm, the ethanol was discarded and the samples air dried for 15 min. DNA in each tube was then resuspended in 300 μL of ddH_20 and 1 mL of Wizard PCR Preps Purification Resin and subjected to a silica extraction to further remove inhibitors. The silica extraction was conducted using the Wizard PCR Preps DNA purification system (Promega) following manufacturer's instructions except each sample was placed in 100 μL of ddH_20. Samples were stored at -20°C in the Ancient DNA Laboratory.

Polymerase Chain Reaction (PCR) and Sequencing

In order to determine a maternal haplotype from the portion of the hypervariable region of the mtDNA genome, each sample was subjected to several PCR reactions. Table 1 and Table

2 display all primers and PCR conditions used. Furthermore, to determine sex of each turkey coprolite a multiplex PCR consisting of primers for the W-linked female specific PstI gene and and the Z-linked ATP synthase gene present in both sexes was conducted with primers modified from D'Costa and Pettite (1998; Table 2).

After PCR amplification, the DNA was visualized by first mixing loading dye with 4 μL of each DNA sample. Loading dye and each sample was placed in separate wells on a 5% polyacrylamide gel. A 20 base pair (bp) ladder was added in a separate well in order to assure appropriate length of the targeted DNA. All gels were then placed under an electric current to separate the DNA and gels were stained with ethidium bromide and visualized under UV light. A successful amplification was indicated by a bright band appearing on each gel in the appropriate bp length.

The remaining PCR product was prepped for DNA sequencing by adding 1 U of ExoI/SAP mixture to destroy unincorporated primers and nucleotides remaining in the samples. After remaining nucleotides and unincorporated primers were destroyed, 2 μL of the amplicons was mixed with 3.6 μL of 10 mM primer and sent to Yale University for sequencing in both directions. Each received sequence was then aligned to a turkey reference sequence from GenBank (EF153719) using *Sequencher*. If contamination was suspected due to modern contamination or due to DNA damage in the sequencing data, the sample was replicated three times in order to determine the original sequence. If no consensus was reached the sample was dropped from the analysis.

Palynological Analysis of Turkey Coprolites

Palynological analysis has become a common procedure for interpreting diet of ancient organisms and can been used to address turkey domestication. All palynological analyses of coprolites were conducted in a clean palynological laboratory with sterile equipment. Each coprolite sample was blown with sterile air in order to remove possible outside pollen contaminates. The portions of coprolite samples processed for pollen analysis weighed between 0.143-1.071g and pollen was extracted following procedures in Fry (1976). Two *Lycopodium* tablets (each containing ~12432 *Lycopodium* pollen grains) were added to each sample and the sample immersed in a solution of 0.5% trisodium phosphate. *Lycopodium* tablets were added as a control to each sample and were used to determine relative concentration values of pollen. After one week of reconstitution, each coprolite was screened to remove large particles. Each large fraction was dried and stored in the paleoenvironmental laboratory for further macrofossil testing. The small fraction after screening was then split into two groups, one to test for pollen and another for possible future phytolith analysis. Samples split for phytolith testing were placed in 95% EtOH and stored in WSU's paleoethnobotanical laboratory in College Hall. Samples taken for pollen analysis then underwent standard pollen processing at Washington State University following Erdtman (1943). Samples designated for pollen analysis were rinsed in ~1.5 mL of 99.5% pure glacial acetic acid and placed in 5mL of a 9:1 acetolysis solution consisting of 100% acetic anhydride (CH_3CO) and 98% pure sulfuric acid (H_2SO_4) for 5 minutes. Samples were rinsed in ~3mL of glacial acetic acid, washed in ~3mL of 95% EtOH and stored in vials with glycerine for analysis.

Pollen analysis from each of these samples was conducted under a Nikon compound stereo microscope at 400x. A standard of two hundred grains were identified for each sample

following Barkley (1934) and Kapp (2000). In the counting process, each sample was placed upon clean slides with sterile coverslips in order to reduce the risk of contaminating pollen from the outside environment. Pollen counts were formatted into pollen concentrations for each coprolite sample using the following formula where p is the total number of grains counted in the gram of sample, m is the marker grains counted, e is the number of marker grains added, and w is the weight of the coprolite or sediment:

$$\text{Pollen concentration}=((p/m)*e)/w$$

Pollen Analysis of Soils

In order to assess the possibility that pollen from the surrounding deposit had contaminated the turkey coprolites, soil samples were taken from selected strata in the midden (Table 3). By analyzing the pollen composition present in each layer, contaminating pollen in each turkey coprolite can be recognized.

Aggregates of soils were first broken into smaller fragments and two *Lycopodium* tablets (~12432 each) were then added to 2.5 cc of soil. Ten percent pure HCl was then added to each soil sample followed by three rinses with dH_20. Soil samples were then screened through 150 mesh screen and swirled to get rid of sand particles. Each sample was then subjected to ~3 mL of 48% HF to break up silicates overnight. Each sample was then rinsed 3 times with dH_20 until a neutral pH was achieved. Approximately 3 mL of 1% KOH was added to each sample to further disaggregate each sample and to remove unwanted alkaline-soluble humates. Each soil sample was then taken through acetolysis (described above) and counted using the same methodology for counting pollen from the turkey coprolites.

Data Analysis

Wild turkey population genetic variability was obtained using data collected from modern *M. g. merriami* populations reported in Mock et al. (2002). As the distribution of ancient wild turkey mtDNA lineages is unknown, extant wild turkey lineages were assumed to be similar to ancient turkey populations. Six modern wild turkey populations described in Mock et al. (2002) were compared to the observed ancient turkey population at Turkey Pen Ruin. Speller et al. (2010) characterized the genetic polymorphisms of aHap1 and aHap2 lineages which align to Mock et al. (2002) maternal lineages A and AA. Using these data, a Fisher's exact test was conducted using Arlequin 3.1 (Excoffier 2005) on the counts of each lineage at each site examined in Mock et al. (2002; Table 6). Further all counts obtained in Mock et al. (2002) were pooled for a final analysis of Merriam turkeys in the American Southwest. An alpha value of .05 was chosen as the significance level in this analysis.

Microsoft Excel 2007 was used to address the possibility of pollen contamination. A chi-squared analysis was conducted on pollen concentrations between turkey coprolites and soils following Reinhard et al. (2006; Table 4). I used soil pollen concentrations as the expected value, with the null hypothesis being no significant differences between coprolites and soils. The alpha value was set at $p=0.05$.

Coprolite pollen data was further analyzed by conducting a correspondence analysis (CA) using STATA 9.2 statistical software. Using the multivariate analysis first described by Greenacre (1984), the correspondence analysis is a way of relating measurements of a characteristic to measurements of another characteristic. The CA analysis allows turkey coprolites to be related by their pollen compositions. Due to the variability in pollen compositions between each turkey coprolite, a CA analysis defines dimensions to explain and

identify this variation of pollen. Linear combinations comprise each dimension, while dimensions represent the variation in each pollen type throughout all samples. The CA then ranks each dimension by the amount of variation it explains. In this study only the first two dimensions were significant. This study utilizes a CA to categorize differences in pollen types between each turkey coprolite. These dimensions can then be graphed to depict relationships in these data. In effect, I utilized a CA analysis in this study to infer different pollen compositions among turkey coprolites.

RESULTS

Maternal Haplotypes and Turkey Sexing

Thirty-one of 42 turkey coprolites were successfully amplified for mitochondrial DNA and aligned to the reference sequence. All haplotypes matched those found today among *M. g. merriami*. Any polymorphisms when compared to the reference sequence were noted and used to designate maternal haplotype (Table 5, Table 6). After aligning each sequence, two mitochondrial lineages were identified as present in the turkey coprolites and were consistent with Speller et al. (2010). Twenty-five of the coprolites were identified as Lineage 1 (aHap1) while 6 were identified as descendants of lineage two (aHap2). Further, although DNA from bone has been successfully extracted from other archaeological specimens, the limited number of turkey bones recovered from Turkey Pen Ruin did not provide amplifiable DNA.

Four turkey coprolites, TPC 12, TPC 15, TPC 24, TPRTC 33 contained one or more ambiguous nucleotides during first sequencing. After three sequence repetitions of these coprolites the majority rule was used to resolve the ambiguous nucleotides. Further contamination from modern turkey was unlikely in this study. Previous analyses of modern store bought turkeys have found them to be most closely related to the Mexican turkey (*M. g. mexicana*) and therefore sequencing of coprolites would reveal any modern contaminants. This study revealed no modern contaminants.

Likewise, nuclear DNA needed to determine sex of the birds was not recovered from any of the coprolites. Three modern store bought turkeys were used as a control to test all redesigned primers. Although the sex of the modern controls was not known, three separate PCR and sequencing reactions on each turkey control was conducted to test the effectiveness of the redesigned turkey primers. After three trials, successful amplifications and a consistent sex for

each bird was observed. Following D'Costa and Pettite (1998), two modern controls were male turkeys that exhibited only the ATP synthase gene and one band when visualized on a polyacrylamide gel. One female turkey was observed among the modern controls displaying two bands, one for the PstI gene and another for the ATP synthase gene. Ancient samples, however, failed to amplify for either PstI or ATP synthase gene.

Fisher's Exact Test

This study used modern turkey lineages and their frequencies to represent ancient wild turkey populations. Although this is an assumption to this study, ancient turkey lineages and their frequencies were impossible to determine. Mitochondrial lineages and associated frequencies described in Mock et al. (2002; Table 6) were compared to lineages and frequencies found at Turkey Pen Ruin via a Fisher's exact test. All p-values were significant at the .05 level. All samples contained a p-value of 0.00000 except for one sample from Spanish Peaks, CO with a p-value of .00025. These values indicate that the Turkey Pen Ruin turkey population is significantly different from all modern wild populations characterized by Mock et al. (2002; Table 6). Likewise, when all modern wild Merriam turkeys were pooled across the American Southwest, Turkey Pen Ruin was statistically different and had a p-value of .00000. Thus it is significantly unlikely that turkey coprolites at Turkey Pen Ruin would have been produced by chance from wild Merriam's turkeys entering the site from any of the six populations analyzed by (Mock et al. 2002).

Pollen Analysis of Soils & Turkey Coprolites

Pollen analysis of soils from each stratum revealed a total of 26 pollen taxa. Pollen types represented with greater than 5% among all samples include *Juniperus*, *Salix*, Cheno-Am, Maize, *Pinus* and low spine Asteraceae. Other species are represented marginally throughout each soil sample and do not contribute more than 5% among all pollen types (Figure 3).

A total of 27 out of 28 coprolites were successfully isolated for pollen. One sample contained tracer spores (i.e. *Lycopodium*) indicating adequate methodology but was missing all other pollen. Thus this sample was excluded from all further analyses. Twenty pollen types were observed in the coprolite samples. Five pollen types among the turkey coprolites were recorded in concentrations exceeding 5%: *Salix*, *Juniperus*, Maize, Poaceae, and low spine Asteraceae (Table 4). Table 4 depicts pollen concentrations calculated using the standard formula (*See Pollen Analysis of Turkey Coprolites)* and used in the chi-squared analysis. A chi-squared analysis revealed a *P*-value of 1.18×10^{-5} that was significant at the 0.001 level. Soil coprolites therefore exhibit significantly different pollen frequencies than those observed in the turkey coprolites.

Further a CA analysis defined two dimensions that composed over 50% of the total variation among the coprolites and among the 20 dimensions analyzed by the CA analysis, only these two were significant. Within the CA analysis, two dimensions explained a total of 51.35% of the variation among all samples. Dimension one explained 31% of the variation and was consistent with the presence of high maize concentrations among the turkey coprolites. Among turkey coprolites examined in this study, all except three coprolites contained maize pollen. Sample TPC-03 contained the highest concentration of maize (91%) and was classified as the aHap2 lineage. Dimension two explained 20.35% of the variation among all coprolites and was

composed of those samples with high levels of Asteraceae high spines. One coprolite, TPC-08, exhibited 42% Asteraceae high spines while most other samples contained trace amounts of this pollen type.

A CA analysis revealed no observable groupings in pollen composition between turkey coprolites and maternal lineage of each turkey coprolite (aHap1 or aHap2; Figure 1). Thus although variation among the samples exists, no significant groupings can be visualized within the coprolites themselves, regardless of lineage. Thus there are no general groups along maternal lineages of turkey coprolites among those analyzed.

DISCUSSION

Turkey Coprolite Contamination

A variety of procedures were conducted in order to reduce and eliminate contamination during DNA extraction and pollen analysis. All samples were extracted in the ancient DNA clean room at WSU College Hall. Portions of each coprolite were used for the extraction of aDNA and each coprolite was only exposed for a short time in the ancient DNA clean room in order to reduce contamination. After extraction each sample was then taken to a separate room for DNA amplification and sequencing, thus further removing the likelihood of modern contamination in each ancient sample.

In order to address the diets of prehistoric turkey from Turkey Pen Ruin, pollen contamination from surrounding environments needs to be recognized. Although the preservation among Southwestern sites is generally high, the possibility for contamination and mixture throughout the midden is also high. In order to determine mixing among the midden column, soil samples were taken from selected strata and analyzed for pollen (Table 3). Further I compared pollen concentrations in the soil with pollen concentrations found in the turkey coprolites. A chi-squared analysis indicated significant differences between the pollen compositions found within the soil and pollen compositions in the turkey coprolites. Although mixing is a common problem in middens, primarily due to bioturbation (Davidson et al. 1999, Davidson 2002), repeated dumping of liquid materials and differential pollen movement (Davidson et al. 1999), this study does not indicate the probability of significant pollen contamination of the turkey coprolites. Therefore, the turkey coprolites can provide reliable information about the turkey diet which is used in this study to infer domestication.

Genetics and Turkey mtDNA

Although zooarchaeological studies are commonly used to address animal domestication, turkey remains are scarce during the Basketmaker II time period, thus other methods are needed to infer domestication (Munro 2006). The use of molecular studies in revealing past domestication processes is well noted. Although one indication of domestication is an alteration of the sex ratio of populations, the use of nuclear DNA from turkey coprolites was not possible in this study. Nonetheless, the use of mitochondrial DNA (mtDNA) revealed two distinct lineages, aHap1 and aHap2 among the coprolites at Turkey Pen Ruin. Speller et al. (2010) argued for aHap1 as a domestic lineage out of 13 other reported turkey lineages in the American Southwest (Szalanski et al. 2000). In order to determine the likelihood that turkeys of only aHap1 and aHap2 were randomly depositing feces at Turkey Pen Ruin, a comparison between six sites across the American Southwest and Turkey Pen Ruin was conducted. Likewise, a comparison between all Merriam turkey lineages, found by Mock et al. (2002), was compared to the lineages present at Turkey Pen Ruin. After calculation, a Fisher's exact test revealed significant differences between Turkey Pen Ruin and all six sites observed by Mock et al. (2002). Further a significant difference was observed when all Merriam's turkeys were summed across all six sites. All p-values were less than .05 indicating the Turkey Pen Ruin is significantly different from at least six modern wild turkey populations. In other words, it is highly unlikely that wild turkey populations were randomly depositing feces at Turkey Pen Ruin.

There are multiple scenarios that would create a significant deviation from a wild population. First, prehistoric people hunted for game, including the turkey (Kantner 2004, Plog 2008). Although unlikely, during these hunting trips, populations of turkey could have been hunted and brought to the site, leading to the presence of a small number of feces belonging to

the hunted turkey. Further wild populations of turkey could have wandered into the site leading to a small number of feces belonging to the wandering wild turkey. In this analysis and Speller et al. (2010), aHap2 is present in small numbers and at first glance might seem to be a wild lineage; however the coprolite analysis in this study implies an undistinguishable diet between the domestic lineage identified in Speller et al. (2010) aHap1 and the other potentially wild lineage aHap2. Thus, if aHap1 is truly representative of a domestic bird, the pollen data presented here are consistent with aHap2 also representing a domestic lineage.

A second scenario, which would explain deviations from wild populations, involves the capture and selective breeding of turkey populations or what Bokonyi (1969) describes as the animal breeding stage. During turkey husbandry, individuals with favorable attributes are isolated and bred to increase the frequency of that characteristic in the next generation. Despite the original genetic variability among turkey populations, the continuance of selective breeding, even though not direct on phenotypes possibly influenced by mtDNA variation, would over time result in the formation of reduced maternal lineages. Reduction of genetic variability, especially in the maternal line, would then display significant differences when compared to wild populations.

Lastly, although a deviation from wild turkey populations was calculated in this study, expected lineages and their frequencies were taken from modern populations. It is possible that ancient wild turkey populations around Turkey Pen Ruin only consisted of these two lineages. If this is the case, given accurate ancient population lineages and their frequencies deviations from wild populations may not exist in a multinomial probability calculation. However, modern lineages of *M. g. merriami* from a single site, from across the American Southwest always exhibit more than 2 lineages (Mock et al. 2002). For example: four lineages, what Mock et al.

(2002) term mitotypes, are present in Stoneman, Arizona, roughly 225miles Southwest of Turkey Pen Ruin. Spanish Peaks, Colorado, roughly 260 miles to the East of Turkey Pen Ruin also contains four distinct lineages. Further, the aHap2 lineage is the most frequent lineage at both Spanish Peaks, CO and Stoneman, AZ. This suggests that although modern lineages and their frequencies were used in this analysis, the presence of only two lineages among the coprolites from a single site, Turkey Pen Ruin, may not be indicative of ancient wild populations. Therefore Turkey Pen Ruin inhabitants could have been specifically isolating and domesticating aHap1 and aHap2 birds which would result in a deviation in a multinomial probability calculation.

Nonetheless, there is little genetic variability in turkey maternal lineages at Turkey Pen Ruin and this observation is not likely the product of birds randomly wandering into the site and depositing their feces. Turkey Pen Ruin is a known human habitation site and therefore the turkeys whose coprolites were studied in this analysis were likely involved with or at least in contact with prehistoric people. Therefore, although selective breeding of turkeys by prehistoric people cannot be unequivocally proven, the minimal genetic variability found in this study in conjunction with a multinomial probability analysis, intimates controlled and selective breeding by human populations or an animal breeding stage, as described by Bokonyi (1969).

Diet and Plant Use at Turkey Pen Ruin

This study also provides dietary evidence of turkey domestication at Turkey Pen Ruin. A pollen analysis reveals a wide variety of plant species eaten by prehistoric turkeys. If turkeys were domesticate at Turkey Pen Ruin, then human populations would have had to feed or control the feeding of their livestock. Previous molecular analyses have identified a single domestic

turkey lineage at Turkey Pen Ruin, aHap1 (Speller et al. 2010). Therefore if this lineage, aHap1, is domesticated, these coprolites should exhibit significant dietary differences than wild turkey coprolites, represented here as being produced by aHap2 turkeys. Dietary reconstructions of both lineages present at Turkey Pen Ruin, aHap1 and aHap2, reveal no significant differences in dietary consumption among the turkey coprolites. Thus if aHap1 exhibits the diet of a domesticated bird, then aHap2 also illustrates a domestic turkey diet. Interestingly, two coprolite samples reported as the 'wild' lineage, aHap2, in Speller et al. (2010), aHap2 contain two of the highest levels of cultivated maize. Because of this, it is likely that both lineages present at Turkey Pen Ruin, aHap1 and aHap2, are domestic turkey lineages.

Further, comparisons to human population diets provided by Aasen (1984) from Turkey Pen Ruin reveal substantial similarities with turkey diet observed in this study. Aasen (1984) observed 23 different pollen taxa in human coprolites from Turkey Pen Ruin, only eight of these pollen taxa were not observed in the turkey coprolites in this study. Aasen (1984) illustrated that Basketmaker II people used many different plant species and thus the presence of the pollen from these plants in archaeological sites is expected. There is no doubt that maize (*Zea mays* ssp. *mays*) was present across the landscape during Basketmaker II. Maize was used as a storable resource and consumed all year long and could have been used to feed turkeys.

Maize pollen found within the turkey coprolites is expected. In this study only three turkey coprolites exhibited no maize pollen, with the remaining coprolites containing from 1%-91% maize pollen grains. Turkey coprolites with high levels of maize could be the result of ingested corn silks or inflorescences. The domestication of the turkey, however, cannot be inferred solely on the presence of maize pollen due to the scavenging abilities of turkeys. Both

wild and domestic turkeys could have had access to these maize resources and therefore maize pollen alone is not a reliable indicator of domestication.

Juniperus is another pollen type which is present in both human (Aasen 1984) and turkey diet at Turkey Pen Ruin. *Juniperus* (Juniper) composed over 28% of total pollen found within the turkey coprolites and had multiple uses by Basketmaker II peoples. Juniper wood was used for a variety of materials such as medicine, basketry, torches, dyes, and kindling (as cited by Aasen 1984). Medicine from Juniper wood with adhering pollen grains would be enough to incorporate *Juniperus* pollen into the turkey and human feces. Likewise, *Juniperus* pollen is produced in high quantities during the winter and the high abundance of *Juniperus* in both human and turkey coprolites suggests a later occupation of both human and turkey populations at Turkey Pen Ruin.

Likewise, *Pinus* spp. was widely utilized by prehistoric people. *P. ponderosa* and *P. edulis* were both present around Turkey Pen Ruin. *P. ponderosa* could have been found at elevations above the site and was primarily used for fire wood however some evidence suggests ingestion of *P. ponderosa* seeds by native inhabitants (as cited by Aasen 1984). Ingestion of nuts could lead to the incorporation of Pine pollen in the diet. Furthermore, by burning Pine wood, pollen could have been released in the air and been ingested by human and turkey populations. Pinyon nuts from *P. edulis* were also commonly used foods by indigenous habitants and are a major component of the macrofossil assemblages at Turkey Pen Ruin (as cited by Aasen 1984, Lepofsky 1986). Therefore, due to the abundance of this food resource and its use by humans, the presence of this pollen type among the turkey coprolites and the soil samples is not surprising.

In this study, 6% of the total pollen found in the turkey coprolites was Cheno-Am pollen. Aasen (1984) reported that Cheno-Am pollen was the most abundant pollen type in the Turkey Pen Ruin human coprolites. These plants include *Chenopodium* spp., *Amaranthus* spp. and *Atriplex* spp., which had several uses for past populations. *Chenopodium* and *Amaranthus* were frequently cooked with maize (Aasen 1984). Furthermore, chenopod leaves were found in the macrofossil analysis at Turkey Pen Ruin suggesting a high amount of Cheno-Am use at Turkey Pen Ruin by prehistoric inhabitants (Aasen 1984). Due the abundance and use of Cheno-Ams by prehistoric people, it is likely that this crop would have been used as a food resource for any domesticated animal like the turkey.

Turkey coprolites also exhibited the presence of other human cultivated species. Other plant remains recovered in previous analyses at Turkey Pen Ruin include sunflower seeds (*Helianthus*), Indian rice grass (*Oryzopsis*), Beeweed (*Cleome*) and prickly pear cactus (*Opuntia* spp.) (Aasen 1984, Lepofsky 1986). Sunflower pollen is classified as high spine Asteraceae and was found in negligible amounts in the soil analysis of this study. Nonetheless, turkey coprolites analyzed in this study reveals 3% high spine Asteraceae overall. Therefore, plants like the sunflower were likely consumed by the turkeys.

Indian rice grass (*Oryzopsis*) and other species likewise were used among Southwestern inhabitants and turkeys. Turkey coprolite pollen reveals close to 8% Poaceae pollen, indicating the direct consumption of grass pollen by prehistoric turkeys. Uses for Indian rice grass include seeds for food and matting for houses and roofs (Moerman 2008). Indian rice grass pollen is included in the Poaceae family and pollen grains counted in the analysis could, in part, represent this species. Although numerous species are classified into this family, the soil analysis from the midden reveals very little Poaceae pollen. This could be due to the way in which Indian rice

grass was processed or the season in which the soils samples were taken. Moerman (2008) describe that Indian rice grass was dropped near the fire, where the seeds would fall off onto a grinding stone. Seeds were then ground and made into cakes. Therefore, the subsequent processing of this grass could be an explanation for why the soil samples do not reveal high levels of Poaceae pollen. Turkeys on the other hand could have directly ingested plant material with adhering pollen grains leading to the frequency of this pollen type in the turkey coprolites. Regardless it is clear that grasses were a major food resource for turkeys at Turkey Pen Ruin.

Another economic plant heavily utilized by turkeys in this study and by Basketmaker II people is Beeweed (*Cleome*). Generally amongst the turkey coprolites examined in this study, Beeweed pollen occurs at an overall frequency of 4%. Therefore, turkeys could have been ingesting leftover flowers collected for consumption by human populations. Aasen (1984) reports high levels of Beeweed pollen found within the human coprolites. She attributes this high level of pollen to the direct ingestion of Beeweed flower. Furthermore, macrofossil analysis revealed whole seeds of *Cleome* in the human coprolites. In contrast, soil samples from the midden column reveal very little Beeweed pollen. *Cleome* pollen is not produced in high numbers in the plant, therefore the presence of high levels of this pollen in both human and turkey coprolites are further evidence of direct consumption of human feces by turkeys or of direct consumption of this plant (Reinhard et al. 2006).

Prickly pear (*Opuntia* sp.) is another resource that would have been used by turkeys and Basketmaker people. Turkeys could have ingested any part of the fruit or cacti pad perhaps containing pollen grains, allowing the addition of this pollen into the coprolites. In this study however only a few samples exhibited one pollen grain of this type. Nonetheless, human coprolites analyzed by Aasen (1984) and turkey coprolites analyzed in this study had similar

concentrations of prickly pear pollen. Human populations would have been able to harvest and consume flowers with high pollen concentrations during the spring, fruits of this plant during the summer months and eat pads of the prickly pear throughout September and October (Cordas 2000, Moerman 2008). Low frequencies of prickly pear pollen in the turkey coprolites likely excludes the possibility that the turkeys were eating flowers in the spring and could therefore suggest a site occupation at a different time of the year. Aasen (1984) and Lepofsky (1986) argue for a late summer/early fall human occupation at Turkey Pen Ruin. Based upon the concentrations of prickly pear pollen it is likely that turkeys were also present at Turkey Pen Ruin throughout late summer and winter.

Both Aasen (1984) and Lepofsky (1986) agree on a late summer/early fall human occupation for Turkey Pen Ruin. The turkey coprolites examined in this study, could also represent turkey droppings from late summer/early fall, however it is also possible that turkeys were simply entering the site before and after human occupation and living off of the refuse after human abandonment. Another scenario which could explain a lack of differences in diet between both lineages, is the possibility that aHap1 is a domestic lineage while aHap2 turkeys were not domesticated but wandering into the site after human occupation and eating leftover materials. Further, the consumption of human feces by the turkeys would create significant similarities between human and turkey pollen which could be misinterpreted as turkey domestication. Despite these scenarios, human populations at Turkey Pen Ruin influenced the diet of nearby or domesticated turkey populations either through direct feeding and animal breeding or through leftover human refuse at the site.

Turkey Domestication at Turkey Pen Ruin

Although the state of an animal species as domestic is difficult to determine, there is a multitude of support for turkey domestication in the American Southwest during the Basketmaker II time period. Not only have past studies theorized the domestication of the turkey in the northern American Southwest (Breitburg 1985, Breitburg 1988, Breitburg 1993, Mock et al. 2002, Ferg 2007, Speller et al. 2010), this study provides molecular and dietary support for two domestic turkey lineages (aHap1 and aHap2) at Turkey Pen Ruin, during the Basketmaker II time period. The probabilities of obtaining coprolites from only two lineages in the frequencies recorded at Turkey Pen Ruin were lower than 0.001. Thus it is unlikely that birds were randomly entering the site, eating human refuse and depositing their feces and likely represents an animal breeding stage is being conducted by human inhabitants of Turkey Pen Ruin.

Dietary reconstructions based upon palynological analysis also reveal a close association between humans and turkeys at the site. This study found a high frequency of known cultivated crops in turkey coprolites and a previous analysis of human coprolites (Aasen 1984) indicates a similar diet between humans and turkeys at Turkey Pen Ruin. Further, pollen evidence suggests the turkey coprolites could have been created during the same part of the year that humans were occupying the site. Although the seasonality of coprolite deposition cannot be unequivocally determined and other scenarios for the similarity in turkey and human diet at Turkey Pen Ruin were discussed, a multinomial probability suggests that wild turkey populations were not randomly entering the site and eating leftover human refuse. Thus it is probable that turkeys and humans were co-habitating at the site.

Soil samples from Turkey Pen Ruin analyzed as an environmental control, indicate that contamination to the turkey coprolites analyzed is minimal. Further, dietary reconstructions of

the domestic lineage reported in previous analyses, aHap1 (Speller et al. 2010) was undistinguishable from another turkey lineage found at Turkey Pen Ruin, aHap2. An animal breeding stage requires care and control in feeding of the domesticated animal, thus aHap1, being a domestic lineage, displays the diet of a domesticated bird. aHap2 diet is indistinguishable from aHap1 diet and therefore it is likely that aHap2 is also a domestic turkey lineage.

The primary aim of this study was to determine if turkey coprolites obtained from a midden column at Turkey Pen Ruin were created from domesticated or wild birds. Due to the paucity of turkey bone at the site, a traditional zooarchaeological approach to the study of turkey domestication was impossible. Using a combination of molecular biology and palynology, the question of turkey domestication was addressed. This interdisciplinary approach allows for the conclusion that the turkey coprolites at Turkey Pen Ruin were made by domesticated birds of lineages aHap1 and aHap2. It should be noted that this conclusion does not address the human use of the turkeys during Basketmaker II but rather focuses solely on the question of turkey domestication at Turkey Pen Ruin during Basketmaker II. Future analyses incorporating an increasing use of molecular tools will have the benefit of both determining the use of these domesticated birds and providing further support for turkey domestication among Basketmaker II people in the Cedar Mesa region.

Table 1. Sequencing primers used for mtDNA of turkey coprolites with annealing temperatures as adopted from Speller et al. (2010). Fifteen microliter PCR reactions contained: 2.4mM DNTPs, 1X PCR Buffer, 0.45mM $MgCl_2$, 0.18μM primer, .3U of Platinum *Taq* (Invitrogen) and 1.5μL of template DNA. Sixty cycles of PCR were conducted as follows: 3 min denaturing at 94°C, followed by 15 second holds at 94°C, at the annealing temperature, and at 72°C, followed by a final 3 min extension period at 72°C.

Target Region*	Primer	Coordinates[†]/ Sequence (5' to 3')	Annealing Temperature
D-Loop 1	T15533F	15433-15533 GTTGTTCTCAACTACGGGAAC	60°C[‡]
	T15750R	15730-15750 GTAGTCATAGGGAGAAATGG	
D-Loop 1A[§]	T15533F	15433-15533 GTTGTTCTCAACTACGGGAAC	55°C
	T15656R	15634-15656 GTATGTGGTATATAAATGTATCG	
D-Loop 1B[§]	T15612F	15612-15633 GGGGTATACTATGCATAATCGT	55°C
	T15750R	15730-15750 GTAGTCATAGGGAGAAATGG	
D-Loop 2	T15709F	15709-15729 ACGGACATAACAACCTTTACC	60°C[‡]
	T15894R	15875-15894 TCTGGTACGTCGAGCATAAC	
D-Loop 3	T15853F	15853-15874 CTTACTGTACTTACCCCATTTG	60°C[‡]
	T16032R	16014-16032 TCGACCGAGGAACCAGAGG	

[†]Coordinates, numbered according to the reference sequence (Genbank Accession Number: AF486875)

[‡]Touch-down PCR used, decreasing the annealing temperature 0.1^{O} C after each cycle.

[§]For the samples degraded below 200 bp, the D-Loop 1 amplicon had to be amplified and sequenced in two smaller fragments.

Table 2: Sequencing primers used for sexing of turkey coprolites modified from (D'Costa and Petitte 1998). Fifteen microliter PCR reactions contained: 2.4mM DNTPs, 1X PCR Buffer, 0.45mM $MgCl_2$, 0.18µM primer, .3U of Platinum *Taq* (Invitrogen) and 1.5µL of template DNA. Sixty cycles of PCR were conducted as follows: 3 min denaturing at 94°C, followed by 15 second holds at 94°C, at the annealing temperature, and at 72°C, followed by a final 3 min extension period at 72°C.

Target Region	Coordinates†/ Sequence (5' to 3')	Annealing Temperature	Amplicon length (bp)
PstI	CAGGAAATGCCAGTTTTATCG	55°C	177
	ATGTTTTGGGGGCAAAAATCC		
ATP Synthase Gene	CTCCATCACTGATGGACAG	55°C	198
	GTAGAACAGCTCAGTTTCCAAG		

Table 3: Pollen analysis of soil data taken from different strata at Turkey Pen Ruin, Utah. A total of 8 soil layers were analyzed for pollen analysis in this study. Each stratum corresponds to a soil layer depicted in Figure 1 and excavated as part of the Cedar Mesa Project.

Stratum	Fraxinus	Salix	Juniperus	Poaceae	Zea mays	Cheno-Am	Sarcobatus	Cleome	Quercus	Artemisia	Asteraceae high spine	Asteraceae low spine	Boerhaavia	Pinus	Celtis	Pseudotsuga	Eriogonum	Populus	Ephedra	Cylindropuntia	Fabaceae	Dalea	Indeterminate	Total
B2 Face	0	38	55	3	10	19	1	0	2	2	2	51	0	4	0	0	0	13	2	2	0	0	9	213
A3	1	0	112	1	8	14	1	0	0	3	1	72	1	10	1	1	1	12	1	6	0	0	16	262
A6	0	33	27	4	21	18	0	1	1	9	1	81	0	7	0	0	1	7	0	0	7	0	13	231
B2	0	14	68	25	8	7	0	1	4	5	2	44	0	9	2	0	1	9	1	0	4	3	14	221
B4	0	4	55	19	18	18	2	0	0	7	5	27	1	25	0	0	1	9	0	1	0	0	14	206
B5	0	89	28	2	17	14	0	0	3	5	1	10	0	16	0	0	0	8	0	0	0	0	7	200
C26	0	0	54	18	18	11	0	0	8	11	1	31	0	33	0	0	1	11	0	0	0	0	25	222
D1	0	12	33	4	5	12	1	5	14	4	3	66	0	15	0	0	4	3	0	3	0	0	16	200

Table 4: Percentage of each pollen type and concentration values of each pollen type used in the Chi-squared analysis for both soils and coprolites. Concentrations were calculated following Reinhard et al. (2006). Soil concentrations were used as expected values and the probability of significance was set at 0.05. Only pollen types present in the soil were used in this analysis.

	Salix	*Juniperus*	Poaceae	*Zea mays*	Cheno-Am	*Sarcobatus*	*Cleome*	*Quercus*	*Artemisia*	Asteraceae high spine	Asteraceae low spine	*Boerhaavia*	*Pinus*	*Celtis*	*Eriogonum*	*Populus*	*Fraxinus*	*Ephedra*	*Cylindropuntia*	*Fabaceae*	*Dalea*
Concentration in Coprolite	6501	10643	2964	4345	1456	1246	1571	343	1546	1234	3187	190	470	25	38	407	0	0	0	0	0
Concentration in Soil	1804	4103	721	997	1073	47	66	303	436	151	3628	18	1120	28	85	873	9	37	113	104	28
% Pollen Coprolites	19	24	7	15	5	0	5	0	4	2	8	0	1	0	0	0	0	0	0	0	0
% Pollen Soil	10	24	4	5	6	0	0	1	2	0	21	0	6	0	0	4	0	0	0	0	0

Table 5: Results of Sequence analysis of Turkey Coprolites from Turkey Pen Ruin.

Sample ID	Weight extracted (g)	15575	15604	15677	15679	15708	15735	15736	15745	15756	15778	15782	15793	15808	15826	15845	15953	Sequence Read	haplotype	Provenience within the midden	DNA Study
Reference (AF486875)		C	C	T	C	G	T	C	G	G	C	C	C	T	G	C	T				
TPC-01	0.81	.	.	.	.	.	C	.	.	.	.	.	.	C	.	.	C	15554-16013	aHap1	Test Pit, Feature A pothole (fill removal)	Speller et al. 2010
TPC-02*	0.16	.	.	.	.	.	C	.	.	.	.	.	.	C	.	.	C	15554-16013	aHap1	Test Pit, Feature A pothole (fill removal)	Speller et al. 2010
TPC-03*	0.18	T	.	C	T	.	.	.	.	.	.	T	T	C	.	T	C	15554-16013	aHAp 2	Test Pit, Feature A pothole (fill removal)	Speller et al. 2010
TPC-04*	0.31	.	.	.	.	.	C	.	.	.	.	.	.	C	.	.	C	15554-16013	aHap1	Test Pit, Feature A pothole (fill removal)	Speller et al. 2010
TPC-05*	0.24	.	.	.	.	.	C	.	.	.	.	.	.	C	.	.	C	15554-16013	aHap1	Test Pit, Feature A pothole (fill removal)	Speller et al. 2010
TPC-06*	0.3	.	.	.	.	.	C	.	.	.	.	.	.	C	.	.	C	15554-16013	aHap1	Test Pit, Feature A pothole (fill removal)	Speller et al. 2010
TPC-07*	0.17	.	.	.	.	.	C	.	.	.	.	.	.	C	.	.	C	15554-16013	aHap1	Test Pit, Feature A pothole (fill removal)	Speller et al. 2010
TPC-08*	0.14	.	.	.	.	.	C	Y	.	.	.	.	.	C	.	.	C	15554-16013	aHap1	Test Pit, Feature A pothole (fill removal)	Speller et al. 2010
TPC-09*	0.24	.	.	.	.	.	C	.	.	.	.	.	.	C	.	.	C	15554-16013	aHap1	Test Pit, Feature A pothole (fill removal)	Speller et al. 2010
TPC-10	0.08																		N/A	No provenience, Test pit	This study

Sample ID	Weight extracted (g)	15575	15604	15677	15679	15708	15735	15736	15745	15756	15778	15782	15793	15808	15826	15845	15953	Sequence Read	haplotype	Provenience within the midden	DNA Study
TPC-11	0.1																		N/A	No provenience, Test pit	This study
TPC-12*	0.27	.	.	.	.	.	C	.	.	.	.	.	.	C	.	.	C	15554-16013	aHap1	No provenience, Test pit	Speller et al. 2010
TPC-13*	0.18	.	.	.	.	.	C	.	.	.	.	.	.	C	.	.	C	15554-16013	aHap1	No provenience, Test pit	Speller et al. 2010
TPC-14*	0.33	T	.	C	T	.	.	.	.	.	.	T	T	C	.	T	C	15554-16013	aHAp 2	No provenience, Test pit	Speller et al. 2010
TPC-15*	0.24	T	.	C	T	.	.	.	.	N	.	T	T	C	.	T	C	15554-16013	aHAp 2	No provenience, Test pit	Speller et al. 2010
TPC-16*	0.23	.	.	.	.	.	C	.	.	.	.	.	.	C	.	.	C	15579-16013	aHap1	No provenience, Test pit	Speller et al. 2010
TPC-17*	0.25	.	.	.	.	.	C	.	.	.	.	.	.	C	.	.	C	15554-16013	aHap1	No provenience, Test pit	Speller et al. 2010
TPC-18*	0.33	.	.	.	.	.	C	.	.	.	.	.	.	C	.	.	C	15554-16013	aHap1	No provenience, Test pit	Speller et al. 2010
TPC-19	0.17																		N/A	No provenience, Test pit	Speller et al. 2010
TPC-20*	0.13	.	.	.	.	.	C	.	.	.	.	.	.	C	.	.	C	15554-16013	aHap1	No provenience, Test pit	Speller et al. 2010
TPC-21*	0.12	T	.	C	T	.	.	.	.	.	.	.	T	C	.	.	C	15554-16013	aHAp 2	No provenience, Test pit	Speller et al. 2010
TPC-22*	0.12	.	.	.	.	.	C	.	.	.	.	.	.	C	.	.	C	15554-16013	aHap1	No provenience, Test pit	Speller et al. 2010
TPC-23*	0.14	.	.	.	.	.	C	.	.	.	.	.	.	C	.	.	C	15554-16013	aHap1	No provenience, Test pit	Speller et al. 2010
TPC-24*	0.09	.	.	.	.	.	C	.	.	.	.	.	.	C	.	.	C	15554-16013	aHap1	No provenience, Test pit	Speller et al. 2010
TPC-25	0.12																		N/A	No provenience, Test pit	This study
TPC-26*	0.09	.	.	.	.	.	C	.	.	.	.	.	.	C	.	.	C	15554-16013	aHap1	No provenience, Test pit	This study
TPC-27*	0.17	.	.	.	.	.	.	.	.	.	.	T	T	C	.	T	C	15554-16013	aHap2	No provenience, Test pit	This study

Sample ID	Weight extracted (g)	15575	15604	15677	15679	15708	15735	15736	15745	15756	15778	15782	15793	15808	15826	15845	15953	Sequence Read	haplotype	Provenience within the midden	DNA Study
TPC-28*	0.09	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	C	15554-16013	aHap1	No provenience, Test pit	This study
PC-29*	0.12	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	C	15554-16013	aHap1	No provenience, Test pit	This study
TPC-30	0.11	.	.	.	.	.	C	.	.	.	.	.	.	C	.	.	C	15554-16013	aHap1	No provenience, Test pit	This study
TPC-31	0.05																		N/A	No provenience, Test pit	This study
TPC-32*	0.09	.	.	.	.	.	C	.	.	.	.	.	.	C	.	.	C	15554-16013	aHap1	No provenience, Test pit	This study
TPRTC-1*1	0.05	T	.	C	T	.	.	.	.	.	.	T	T	C	.	T	C	15580-15998	aHAp 2	Strata D-2	Speller et al. 2010
TPRTC-23	0.27																		N/A	Strata B-4	Speller et al. 2010
TPRTC-29	0.1	.	.	.	.	.	C	.	.	.	.	.	.	C	.	.	C	15554-16013	aHap1		This study
TPRTC-30*	0.34	.	.	.	.	.	C	.	.	.	.	.	.	C	.	.	C	15554-16013	aHap1	Strata A-1	Speller et al. 2010
TPRTC-31	0.34																		N/A	Strata A-1	Speller et al. 2010
TPRTC-33*	0.19	.	.	.	.	.	C	.	.	.	.	.	.	C	.	.	C	15554-16013	aHap1	Strata B-1	Speller et al. 2010
TPRTC-39	0.13																		N/A		This study
TPRTC-54	0.25																		N/A	Strata B-3	Speller et al. 2010
TPRTC-55	0.16																		N/A	Strata B-3	Speller et al. 2010
TPRTC-56	0.08																		N/A		This study

* Samples taken for palynological analysis.

Table 6: Wild modern *Meleagris gallopavo merriami* maternal lineages described by Mock et al. (2002). Values represent the number of individuals present at each location with each maternal lineage. Maternal lineage A described from Mock et al. (2002) aligns with lineage aHap2 described by Speller et al. (2010). Lineage AA described by Mock et al. (2002) corresponds to the lineage aHap1 described by Speller et al. (2010).

Location	Approximate Distance from Turkey Pen Ruin (miles)	Maternal Lineage (Mitotype) from Mock et al. 2002											
		A (aHap2)	B	C	D	E	F	G	H	I	K	P	AA (aHap1)	Total
Colorado Springs,CO	300	8	0	0	1	0	0	0	0	0	0	1	0	10
Spanish Peaks,CO	260	7	1	0	0	0	0	1	0	0	0	0	1	10
Stoneman Lake, AZ	225	7	0	0	2	0	0	0	5	0	1	0	0	15
White Mountain, AZ	400	11	0	0	1	3	3	0	0	2	0	0	1	21
Ruidoso, NM	450	3	1	2	0	0	0	0	1	0	0	0	0	7
Raton Mesa, CO	260	7	0	0	0	0	0	1	0	0	0	1	1	10
All Merriam's Found By Mock et al. 2002		43	2	2	4	3	3	2	6	2	1	2	3	73

Table 7: Pollen counts recorded from turkey coprolites excavated from Turkey Pen Ruin, Utah.

Sample Id	Lycopodium	Salix	Juniperus	Poaceae	Zea mays	Cheno-Am	Sarcobatus	Apiaceae	Cleome	Quercus	Sphaeralcea	Populus	Cirsium-type	Artemisia	Pinus	Asteraceae high spine	Asteraceae low spine	Boerhaavia	Fraxinus	Celtis	Eriogonum	Echinocereus	Indeterminate	Total Pollen Count	Concentration Values
TPC-02	259	84	83	12	19	5	0	0	0	0	0	0	0	1	2	0	0	0	0	0	1	0	5	212	38183
TPC-03	53	1	3	29	175	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	3	212	314734
TPC-04	534	74	106	15	4	7	0	0	2	0	0	0	0	0	0	0	0	1	0	0	0	2	6	217	20494
TPC-05	100	89	60	9	41	7	0	0	0	0	0	0	0	3	1	4	0	0	0	0	0	0	4	218	175415
TPC-06	75	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TPC-07	384	87	76	31	2	5	0	0	0	0	0	0	0	6	0	0	0	1	0	0	0	0	5	213	54085
TPC-08	343	15	35	9	0	4	5	0	0	0	2	0	0	13	0	97	37	0	4	0	0	0	8	229	46111
TPC-09	87	65	135	6	1	2	3	6	20	0	2	18	0	10	0	0	2	0	0	0	0	0	11	281	26769
TPC-12	721	9	57	19	3	25	24	1	10	0	8	9	0	8	1	3	40	0	0	0	0	0	3	220	24712
TPC-13	273	17	48	38	0	22	23	0	7	3	2	20	2	2	0	2	31	0	0	0	0	0	10	227	55726
TPC-14	175	47	24	0	0	4	8	0	10	0	0	0	8	6	1	33	51	7	3	2	0	0	5	209	40511
TPC-15	62	18	13	0	23	10	13	0	3	0	0	0	0	0	0	1	111	12	0	0	1	0	2	207	184474
TPC-16	111	42	103	14	4	8	1	0	7	7	0	1	0	10	11	0	5	0	0	0	0	0	3	216	82849
TPC-17	106	64	95	4	6	2	0	0	34	10	0	0	2	14	2	0	6	0	0	0	0	0	1	240	52563
TPC-18	370	42	70	0	20	15	4	5	10	0	0	0	2	19	0	2	5	2	0	0	0	0	4	200	26987
TPC-20	1210	67	30	60	8	4	3	0	17	0	2	0	0	4	5	5	5	0	0	0	0	1	3	214	12895
TPC-21	548	49	50	11	1	5	1	2	66	16	0	0	0	3	3	1	9	0	0	0	0	0	4	221	58983
TPC-22	857	6	125	11	1	8	5	0	5	1	1	0	0	15	10	5	15	0	0	0	3	0	1	212	12350
TPC-23	175	47	108	7	0	6	3	1	11	4	0	0	0	11	2	2	6	4	0	0	0	0	3	215	50827

Sample Id	Lycopodium	Salix	Juniperus	Poaceae	Zea mays	Cheno-Am	Sarcobatus	Apiaceae	Cleome	Quercus	Sphaeralcea	Populus	Cirsium-type	Artemisia	Pinus	Asteraceae high spine	Asteraceae low spine	Boerhaavia	Fraxinus	Celtis	Eriogonum	Echinocereus	Indeterminate	Total Pollen Count	Concentration Values
TPC-24	57	2	190	1	0	0	0	1	1	3	0	0	0	2	0	0	0	0	0	0	0	0	2	202	248911
TPC-26	27	73	21	25	17	13	13	1	10	2	1	2	0	34	0	0	0	0	0	0	0	0	7	219	751956
TPC-27	976	7	32	29	61	5	7	0	2	4	1	14	0	11	6	14	7	1	0	0	0	2	1	204	33528
TPC-28	996	7	12	8	61	28	34	0	4	0	5	0	0	1	4	1	35	2	0	0	0	0	0	202	35263
TPC-29	581	33	28	52	17	9	5	5	7	3	0	0	0	3	11	7	14	0	5	0	0	0	1	200	59437
TPC-32	365	47	91	15	5	3	7	0	13	1	0	0	0	15	0	10	11	0	0	2	1	0	0	221	41358
TPRTC-11	126	0	14	0	201	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	221	276016
TPRTC-30	341	4	32	58	13	8	6	0	2	0	0	0	0	43	12	3	28	0	0	0	0	0	0	209	51310
TPRTC-33	550	26	32	3	0	24	31	1	0	0	0	0	0	8	3	4	83	0	0	0	0	0	1	216	58123

Figure 1: Turkey Pen Ruin midden column as excavated in 1973 by R.G. Matson and William D. Lipe and copied from Speller et al. (2010). Midden column is 1.4 m deep and 0.5m wide. Soil strata are depicted in the midden column. Calibrated radiocarbon dates are listed for some strata. The entire column represents approximately 1,000 years of soil deposition.

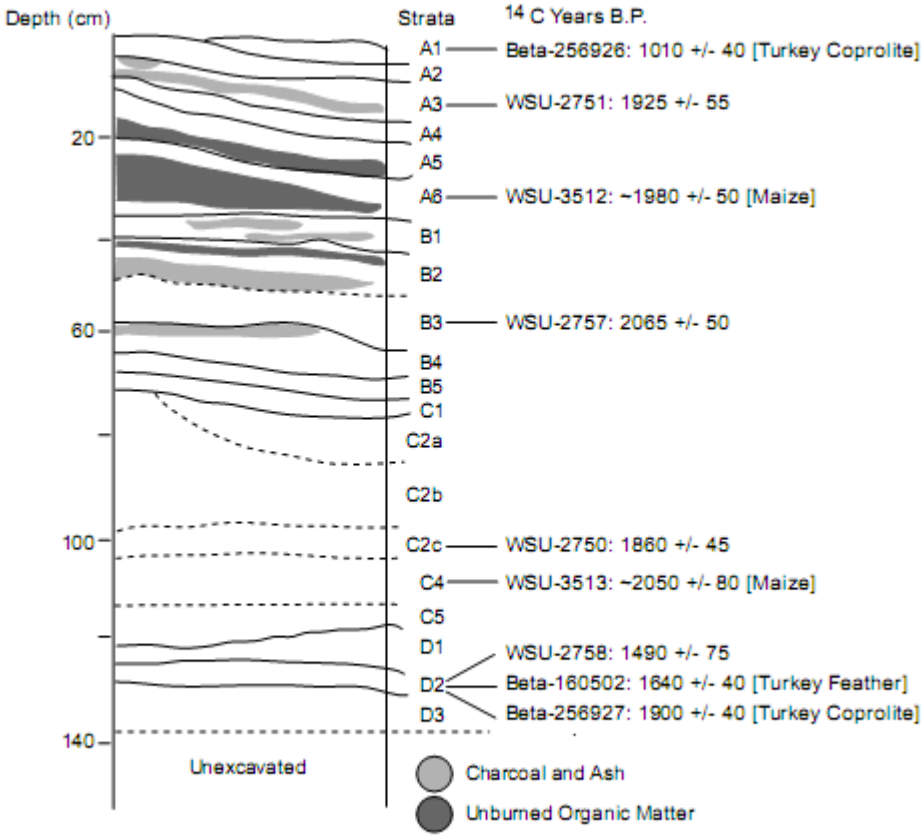


Figure 2: Turkey coprolite lineages displayed as a function of pollen variability. Pollen variability was characterized by dimensions after conduction of a CA analysis. Correspondence analysis (CA) determined two significant dimensions in this study. Dimension 1: Maize Concentration explained 31% of the total variation among all samples. Dimension 2: Asteraceae High Spine Concentration explained 20.35% of the total variation among all samples. No relationship between pollen composition and turkey coprolite lineage were found.

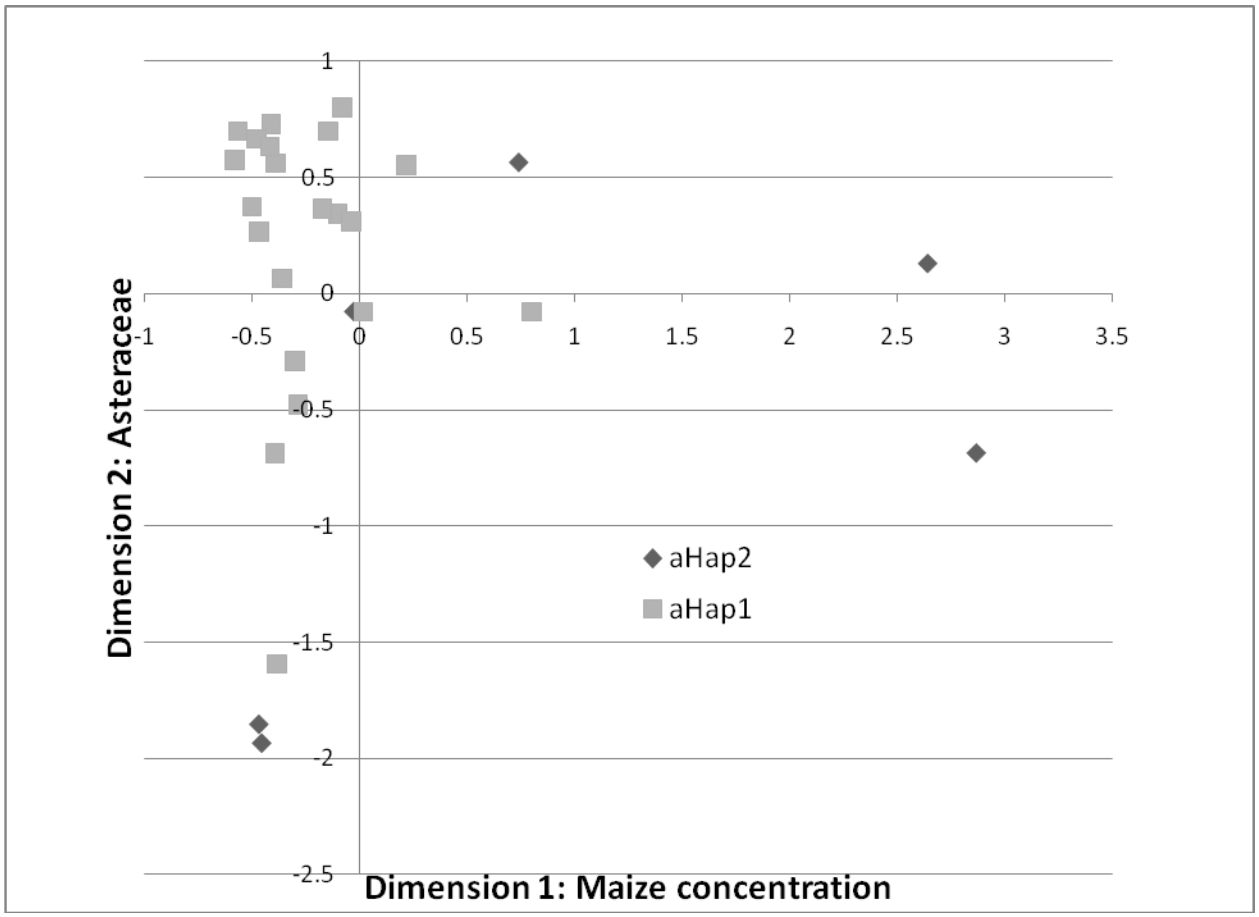
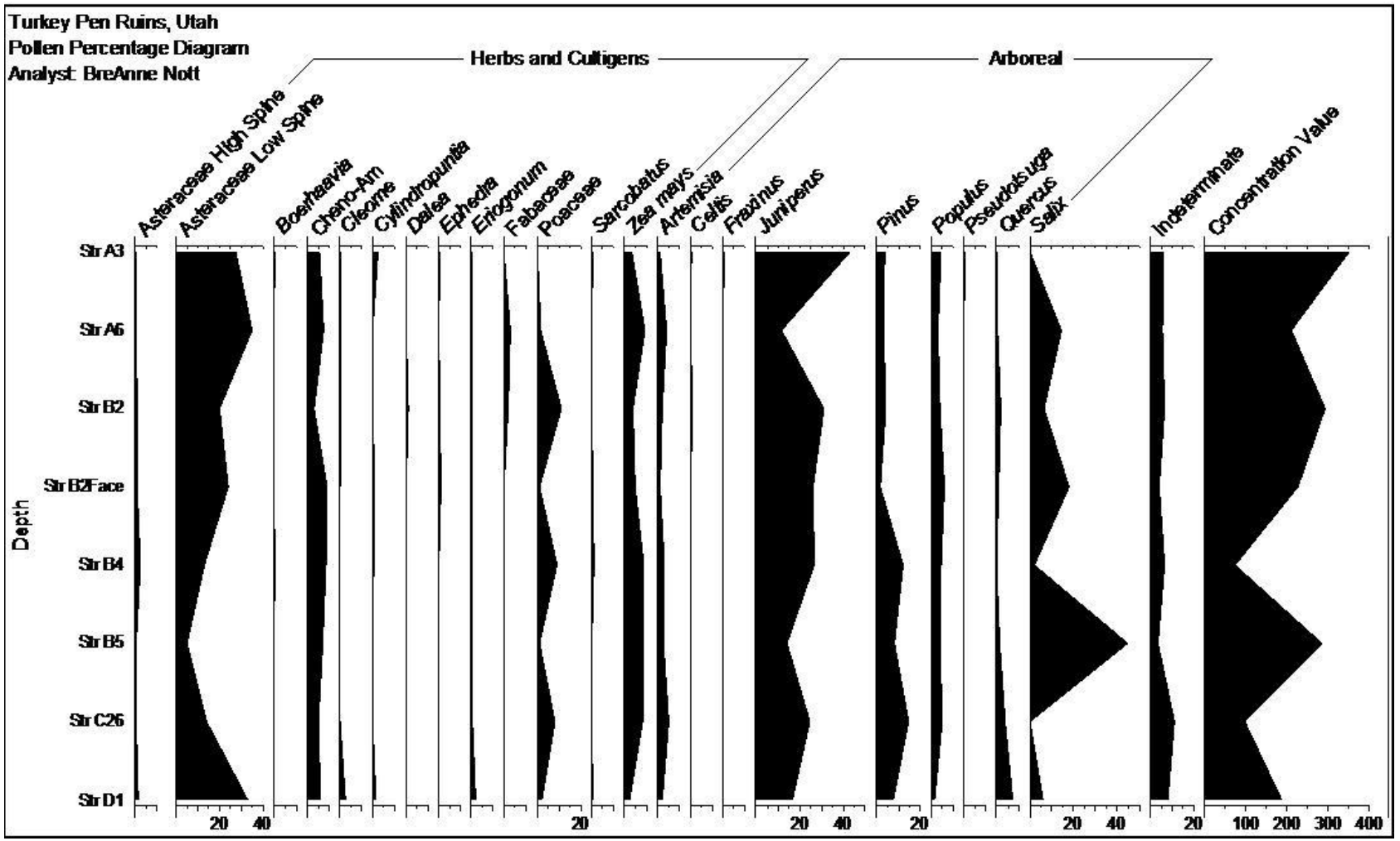


Figure 3: Soil pollen percentage diagram from Turkey Pen Ruin, Grand Gulch, Utah. Samples are placed in stratigraphic order as represented by Figure 1. Due to the presence of two layers in stratum B2 a second sample (B2 Face) was taken and analyzed for pollen.



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Appendices

Appendix 1: Samples collected by Brian M. Kemp 4/21/2009.

Site Number	Sample #	Sample Description	WSU Bag #	WSU Bag Label	CMP Bag	CMP Label	Un/ screened	Strata	Caked/ Loose	Mesh Size	Box
42SA3714	1	Human coprolite	58	Sidebag 1 (from profile)	1	8/10/72 AMNH-70 side B-5	Unscreened	B-5	Loose		33
42SA3714	2	Human coprolite	58	Sidebag 1 (from profile)	1	8/10/72 AMNH-70 side B-5	Unscreened	B-5	Loose		33
42SA3714	3	Human coprolite	58	Sidebag 1 (from profile)	1	8/10/72 AMNH-70 side B-5	Unscreened	B-5	Loose		33
42SA3714	4	Human coprolite	58	Sidebag 1 (from profile)	2	8/9/72 AMNH-70 side A-6	Unscreened	A-6	Loose		33
42SA3714	5	Human coprolite	58	Sidebag 1 (from profile)	2	8/9/72 AMNH-70 side A-6	Unscreened	A-6	Loose		33
42SA3714	6	Human coprolite	58	Sidebag 1 (from profile)	2	8/9/72 AMNH-70 side A-6	Unscreened	A-6	Loose		33
42SA3714	7	Human coprolite	58	Sidebag 1 (from profile)	2	8/9/72 AMNH-70 side A-6	Unscreened	A-6	Loose		33
42SA3714	8	Human coprolite	58	Sidebag 1 (from profile)	2	8/9/72 AMNH-70 side A-6	Unscreened	A-6	Loose		33
42SA3714	9	Possible human coprolite	52	Sidebag 1 (from profile)	1		Unscreened	D-1	Loose		31
42SA3714	10	Possible turkey coprolite	53	Sidebag 1 (from profile)	1		Unscreened	D-1	Loose		31
42SA3714	11	Turkey coprolite	54	Sidebag 1 (from profile)	1		Unscreened	D-2	Loose		32
42SA3714	12	Possible human coprolite	54	Sidebag 1 (from profile)	1		Unscreened	D-2	Loose		32
42SA3714	13	Probable yucca quid	54	Sidebag 1 (from profile)	1		Unscreened	D-2	Loose		32
42SA3714	18	Probable yucca quid	54	Sidebag 1 (from profile)	1		Unscreened	D-2	Loose		32
42SA3714	20	Sediment sample	54	Sidebag 1 (from profile)	1		Unscreened	D-2	Loose		32
42SA3714	14	Possible human coprolite	55	Sidebag 1 (from profile)	1		Unscreened	D-2	Loose		32
42SA3714	15	Possible human coprolite	55	Sidebag 1 (from profile)	1		Unscreened	D-2	Loose		32
42SA3714	16	Possible human coprolite	55	Sidebag 1 (from profile)	1		Unscreened	D-2	Loose		32

Site Number	Sample #	Sample Description	WSU Bag #	WSU Bag Label	CMP Bag	CMP Label	Un/ screened	Strata	Caked/ Loose	Mesh Size	Box
42SA3714	17	Possible human coprolite	55	Sidebag 1 (from profile)	1		Unscreened	D-2	Loose		32
42SA3714	19	Sediment sample	55	Sidebag 1 (from profile)	1		Unscreened	D-2	Loose		32
42SA3714	21	Human coprolite	56	Sidebag 1 (from profile)		C-1 (side clearing)	Unscreened	C-1	Loose		33
42SA3714	22	Human coprolite	56	Sidebag 1 (from profile)		C-1 (side clearing)	Unscreened	C-1	Loose		33
42SA3714	23	Turkey coprolite	57	Sidebag 1 (from profile)	1	8/10/72 AMNH-70 side B-4	Unscreened	B-4	Loose		33
42SA3714	24	Possible human coprolite	57	Sidebag 1 (from profile)	1	8/10/72 AMNH-70 side B-4	Unscreened	B-4	Loose		33
42SA3714	25	Possible human coprolite	57	Sidebag 1 (from profile)	1	8/10/72 AMNH-70 side B-4	Unscreened	B-4	Loose		33
42SA3714	26	Possible human coprolite	57	Sidebag 1 (from profile)	1	8/10/72 AMNH-70 side B-4	Unscreened	B-4	Loose		33
42SA3714	27	Possible human coprolite	56	Sidebag 1 (from profile)		8/10/72 AMNH-70 side B-2	Unscreened	B-2	Loose		33
42SA3714	28	Possible human coprolite	56	Sidebag 1 (from profile)		8/10/72 AMNH-70 side B-2	Unscreened	B-2	Loose		33
42SA3714	29	Turkey coprolite	56	Sidebag 1 (from profile)		8/9/72 AMNH-70 A-1 side	Unscreened	A-1	Loose		33
42SA3714	30	Turkey coprolite	56	Sidebag 1 (from profile)		8/9/72 AMNH-70 A-1 side	Unscreened	A-1	Loose		33
42SA3714	31	Turkey coprolite	56	Sidebag 1 (from profile)		8/9/72 AMNH-70 A-1 side	Unscreened	A-1	Loose		33
42SA3714	32	Human coprolite	59	Sidebag 1 (from profile)	2	8/10/72 AMNH-79 side B-1	Unscreened	B-1	Loose		34
42SA3714	33	Possible turkey coprolite	59	Sidebag 1 (from profile)	2	8/10/72 AMNH-79 side B-1	Unscreened	B-1	Loose		34
42SA3714	34	Human coprolite	60	Sidebag 1 (from profile)	1	8/10/72 AMNH-79 side C-2	Unscreened	C-2	Loose		34
42SA3714	35	Human coprolite	60	Sidebag 1 (from profile)	1	8/10/72 AMNH-79 side C-2	Unscreened	C-2	Loose		34
42SA3714	36	Human coprolite	60	Sidebag 1 (from profile)	1	8/10/72 AMNH-79 side C-2	Unscreened	C-2	Loose		34
42SA3714	37	Human coprolite	60	Sidebag 1 (from profile)	1	8/10/72 AMNH-79 side C-2	Unscreened	C-2	Loose		34
42SA3714	38	Human coprolite	60	Sidebag 1 (from profile)	1	8/10/72 AMNH-79 side C-2	Unscreened	C-2	Loose		34
42SA3714	39	Turkey coprolite	60	Sidebag 1 (from profile)	1	8/10/72 AMNH-79 side C-2	Unscreened	C-2	Loose		34

Site Number	Sample #	Sample Description	WSU Bag #	WSU Bag Label	CMP Bag	CMP Label	Un/ screened	Strata	Caked/ Loose	Mesh Size	Box
42SA3714	40	Human or dog coprolite (mostly hair)	60	Sidebag 1 (from profile)	1	8/10/72 AMNH-79 side C-2	Unscreened	C-2	Loose		34
42SA3714	41	Possible non-human coprolite (dog?)	61	Sidebag 1 (from profile)	1	8/10/72 AMNH-79 side C-2	Unscreened	C-2	Loose		34
42SA3714	42	Probable human coprolite	61	Sidebag 1 (from profile)	1	8/10/72 AMNH-79 side C-2	Unscreened	C-2	Loose		34
42SA3714	43	Probable human coprolite	61	Sidebag 1 (from profile)	1	8/10/72 AMNH-79 side C-2	Unscreened	C-2	Loose		34
42SA3714	44	Probable human coprolite	61	Sidebag 1 (from profile)	1	8/10/72 AMNH-79 side C-2	Unscreened	C-2	Loose		34
42SA3714	45	Probable human coprolite	61	Sidebag 1 (from profile)	1	8/10/72 AMNH-79 side C-2	Unscreened	C-2	Loose		34
42SA3714	46	Probable human coprolite	61	Sidebag 1 (from profile)	1	8/10/72 AMNH-79 side C-2	Unscreened	C-2	Loose		34
42SA3714	47	Probable human coprolite	61	Sidebag 1 (from profile)	1	8/10/72 AMNH-79 side C-2	Unscreened	C-2	Loose		34
42SA3714	48	Probable human coprolite	61	Sidebag 1 (from profile)	1	8/10/72 AMNH-79 side C-2	Unscreened	C-2	Loose		34
42SA3714	49	Probable human coprolite	61	Sidebag 1 (from profile)	1	8/10/72 AMNH-79 side C-2	Unscreened	C-2	Loose		34
42SA3714	50	Probable human coprolite	61	Sidebag 1 (from profile)	1	8/10/72 AMNH-79 side C-2	Unscreened	C-2	Loose		34
42SA3714	51	Possible quid	61	Sidebag 1 (from profile)	1	8/10/72 AMNH-79 side C-2	Unscreened	C-2	Loose		34
42SA3714	52	Probable turkey bone	61	Sidebag 1 (from profile)	1	8/10/72 AMNH-79 side C-2	Unscreened	C-2	Loose		34
42SA3714	53	Strip of rabbit fur	61	Sidebag 1 (from profile)	1	8/10/72 AMNH-79 side C-2	Unscreened	C-2	Loose		34
42SA3714	54	Turkey coprolite	63	Sidebag 1 (from profile)	1	8/10/72 AMNH-79 side B-3	Unscreened	B-3	Loose		35
42SA3714	55	Turkey coprolite	63	Sidebag 1 (from profile)	1	8/10/72 AMNH-79 side B-3	Unscreened	B-3	Loose		35
42SA3714	56	Turkey coprolite	63	Sidebag 1 (from profile)	1	8/10/72 AMNH-79 side B-3	Unscreened	B-3	Loose		35
42SA3714	57	Human coprolite	63	Sidebag 1 (from profile)	1	8/10/72 AMNH-79 side B-3	Unscreened	B-3	Loose		35
42SA3714	58	Human coprolite	63	Sidebag 1 (from profile)	1	8/10/72 AMNH-79 side B-3	Unscreened	B-3	Loose		35
42SA3714	59	Unidentified bone	63	Sidebag 1 (from profile)	1	8/10/72 AMNH-79 side B-3	Unscreened	B-3	Loose		35
42SA3714	60	Unidentified bone	63	Sidebag 1 (from	1	8/10/72 AMNH-79	Unscreened	B-3	Loose		35

Site Number	Sample #	Sample Description	WSU Bag #	WSU Bag Label	CMP Bag	CMP Label	Un/ screened	Strata	Caked/ Loose	Mesh Size	Box
				profile)		side B-3					
42SA3714	61	Turkey bone	64	Sidebag 1 (from profile)	1	8/10/72 AMNH-79 C-2 side	Unscreened	C-2	Loose		35
42SA3714	62	Quid	64	Sidebag 1 (from profile)	1	8/10/72 AMNH-79 C-2 side	Unscreened	C-2	Loose		35
42SA3714	63	Quid	64	Sidebag 1 (from profile)	1	8/10/72 AMNH-79 C-2 side	Unscreened	C-2	Loose		35
42SA3714	64	Human coprolite	64	Sidebag 1 (from profile)	1	8/10/72 AMNH-79 C-2 side	Unscreened	C-2	Loose		35
42SA3714	65	Human coprolite	64	Sidebag 1 (from profile)	1	8/10/72 AMNH-79 C-2 side	Unscreened	C-2	Loose		35
42SA3714	66	Human coprolite	64	Sidebag 1 (from profile)	1	8/10/72 AMNH-79 C-2 side	Unscreened	C-2	Loose		35
42SA3714	67	Human coprolite	64	Sidebag 1 (from profile)	1	8/10/72 AMNH-79 C-2 side	Unscreened	C-2	Loose		35
42SA3714	68	Human coprolite	64	Sidebag 1 (from profile)	1	8/10/72 AMNH-79 C-2 side	Unscreened	C-2	Loose		35
42SA3714	69	Human coprolite	65	Sidebag 1 (from profile)	1	no paper label	Unscreened		Loose		35
42SA3714	70	Human coprolite	65	Sidebag 1 (from profile)	1	no paper label	Unscreened		Loose		35
42SA3714	71	Human coprolite	65	Sidebag 1 (from profile)	1	no paper label	Unscreened		Loose		35
42SA3714	72	Human coprolite	65	Sidebag 1 (from profile)	1	no paper label	Unscreened		Loose		35
42SA3714	73	Human coprolite	65	Sidebag 1 (from profile)	1	no paper label	Unscreened		Loose		35
42SA3714		not examined	51	Sidebag 1 (from profile)	1		Unscreened	D-1	Caked		31
42SA3714		no samples collected	56	Sidebag 1 (from profile)		8/10/72 AMNH-70 A-2 side	Unscreened	A-2	Loose		33
42SA3714		no samples collected	59	Sidebag 1 (from profile)	1	8/10/72 AMNH-70 A-3 side	Unscreened	A-3	Loose		34
42SA3714		no samples collected	62	Sidebag 1 (from profile)	1	8/9/72 AMNH-70 side A-5	Unscreened	A-5	Loose		35
42SA3714		not examined	63	Sidebag 1 (from profile)	2	A-?	Unscreened	A-?	Loose		35
42SA3714		Turkey coprolite									56
42SA3714		Turkey coprolite									56
42SA3714		Turkey coprolite									56
42SA3714		Turkey coprolite									56
42SA3714		Turkey coprolite									56
42SA3714		Turkey coprolite									56

Site Number	Sample #	Sample Description	WSU Bag #	WSU Bag Label	CMP Bag	CMP Label	Un/ screened	Strata	Caked/ Loose	Mesh Size	Box
42SA3714		Turkey coprolite									56
42SA3714		Turkey coprolite									56
42SA3714		Turkey coprolite									56
42SA3714		Turkey coprolite									56
42SA3714		Turkey coprolite									56
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42SA3714		Turkey coprolite									56
42SA3714		Turkey coprolite									56
42SA3714		Turkey coprolite									56
42SA3714		Turkey coprolite									56
42SA3714	Not t-1	Corncob	58	Sidebag 1 (from profile)	1		Unscreened	A-6	Loose		33
42SA3714	Not t-2	Corncob	58	Sidebag 1 (from profile)	1		Unscreened	A-6	Loose		33
42SA3714	Not t-3	Corn stalk	58	Sidebag 1 (from profile)	1		Unscreened	A-6	Loose		33
42SA3714	Not t-4	Corncobs (2)	57	Sidebag 1 (from profile)	1	8/10/72 AMNH-70 side B-4	Unscreened	B-4	Loose		33

Appendix 2: Bone samples collected by BreAnne M. Nott 10/2/2009, 10/6/2009, and 10/9/2009.

Site	Id	Bag #	Un/ Screened	Strata	Caked/Loose	Mesh	Box	Layer (if provided)	Id (if possible)
42SA3714	TPRTB3	168	Screened	B3Face	Loose		57		Turkey Bone
42SA3714	TPRTB4	167	Screened	B4 Face	Loose		57		Bone
42SA3714	TPRTB5	167	Screened	B4 Face	Loose		57		Turkey Bone
42SA3714	TPRTB6	167	Screened	B4 Face	Loose		57		Turkey Bone
42SA3714	TPRTB7	167	Screened	B4 Face	Loose		57		Bone
42SA3714	TPRTB8	56	Unscreened	Side B2	Loose		33	B-2	Bone
42SA3714	TPRTB9	57	Unscreened	Side Bag 1	Loose		33	B4	Bone
42SA3714	TPRTB10	57	Unscreened	Side Bag 1	Loose		33	B4	Bone
42SA3714	TPRTB11	58	Unscreened	Side Bag 1	Loose		33	A6	Bone
42SA3714	TPRTB12	58	Unscreened	Side Bag 1	Loose		33	A6	Bone
42SA3714	TPRTB13	58	Unscreened	Side Bag 1	Loose		33	B5	Bone
42SA3714	TPRTB14	59	Unscreened	Side Bag 1	Loose		34	B1	Bone
42SA3714	TPRTB15	62	Unscreened	Side Bag 1	Loose		35	A5	Bone
42SA3714	TPRTB16	62	Unscreened	Side Bag 1	Loose		35	A5	Bone
42SA3714	TPRTB17	64	Unscreened	Side Bag 1	Loose		35	C2 side	Bone
42SA3714	TPRTB18	65	Unscreened	Side Bag 1	Loose		35	From Profile	Bone
42SA3714	TPRTB19	65	Unscreened	Side Bag 1	Loose		35	From Profile	Bone
42SA3714	TPRTB20	65	Unscreened	Side Bag 1	Loose		35	From Profile	Bone

Appendix 3: Soil samples collected by BreAnne M. Nott 10/2/2009.

Date	Site	Id	Bag Number	Screened/Un	Strata	Caked/Loose	Mesh	Box
10/2/2009	42SA3714	TPRTS1	3	Unscreened	A3	Caked		3
10/2/2009	42SA3714	TPRTS2	11	Unscreened	A6	Caked		5
10/2/2009	42SA3714	TPRTS3	20	Unscreened	B2	Caked		10
10/2/2009	42SA3714	TPRTS4	26	Unscreened	B4	Caked		14
10/2/2009	42SA3714	TPRTS5	31	Unscreened	B5	Caked		18
10/2/2009	42SA3714	TPRTS6	39	Unscreened	C26	Loose		22
10/2/2009	42SA3714	TPRTS7	51	Unscreened	D1	Caked		31

Appendix 4: Selected observations on each bag searched through by BreAnne M. Nott and Dr. William Lipe while collecting bone and soil samples.

Site	Bag Number	Un/Screened	Strata	Caked/Loose	Mesh	Box	Layer	Notes
42SA3714	172	Screened	A3 Face	Loose		57		
42SA3714	170	Screened	A5 Face	Loose		57		Gizzard Stone
42SA3714	169	Screened	A6 Face	Loose		57		
42SA3714	168	Screened	B3 Face	Loose		57		
42SA3714	167	Screened	B4 Face	Loose		57		Gizzard Stone/ Reptile Vertebrae
42SA3714	166	Screened	B5 Face	Loose		57		Gizzard Stone
42SA3714	165	Screened	C5 side	Loose		57		
42SA3714	160	Screened	B2 Face	Loose		57		2 Gizzard Stone
42SA3714	56	Unscreened	Side Bag 1	Loose		33	A1	
42SA3714	56	Unscreened	Side Bag 1	Loose		33	A2	
42SA3714	56	Unscreened	Side Bag 1	Loose		33	A4	
42SA3714	56	Unscreened	Side Bag 1	Loose		33	B2	
42SA3714	56	Unscreened	Side Bag 1	Loose		33	C1	
42SA3714	57	Unscreened	Side Bag 1	Loose		33	B4	
42SA3714	58	Unscreened	Side Bag 1	Loose		33	A6	
42SA3714	58	Unscreened	Side Bag 1	Loose		33	B5	
42SA3714	59	Unscreened	Side Bag 1	Loose		34	B1	
42SA3714	59	Unscreened	Side Bag 1	Loose		34	A3	
42SA3714	60	Unscreened	Side Bag 1	Loose		34	C2	
42SA3714	61	Unscreened	Side Bag 1	Loose		34	C2	
42SA3714	62	Unscreened	Side Bag 1	Loose		35	A5	Gizzard Stone
42SA3714	63	Unscreened	Side Bag 1	Loose		35	B3 Side	
42SA3714	63	Unscreened	Side Bag 1	Loose		35	B3?	
42SA3714	64	Unscreened	Side Bag 1	Loose		35	C2 Side	
42SA3714	65	Unscreened	Side Bag 1	Loose		35	From Profile	