Article:
Speller, Camilla Filomena orcid.org/0000-0001-7128-9903 and Yang, Dongya (2016) Identifying the sex of archaeological turkey remains using ancient DNA techniques. Journal of Archaeological Science Reports. 520–525. ISSN 2352-409X

https://doi.org/10.1016/j.jasrep.2016.05.049

Journal of Archaeological Science: Reports

Identifying the sex of archaeological turkey remains using ancient DNA techniques

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ABSTRACT

Accurate sex identification of archaeological turkey remains is important for deciphering hunting and husbandry practices in pre-contact North America, particularly in the Southwest United States and Mesoamerica where domestic turkeys were raised. Although the sexual dimorphism of turkeys means that relatively complete elements can be distinguished using osteometric approaches, sexing fragmentary or juvenile remains is challenging. Here, we propose a simple and highly-sensitive co-amplification approach which targets highly-repetitive DNA (hrDNA) sequences on the turkey W-chromosome. This technique simultaneously co-amplifies both hrDNA and mitochondrial DNA (mtDNA) fragments: the amplification of the W chromosome identifies the heterogametic sex (females), while the mtDNA fragment acts as an internal positive control to monitor for false negative results. To demonstrate the sensitivity and accuracy of this technique, we applied it to 20 modern turkeys and 117 archaeological turkey bones from 25 sites (ca. AD700-1700), including 32 samples from Sand Canyon Pueblo (AD1250-1300). We amplified ancient DNA from 86% of the ancient remains, demonstrating the sensitivity of the technique for targeting nuclear DNA. The correspondence between morphological size and the genetic sex identification for 100% of the complete skeletal elements demonstrates the accuracy and robusticity of this approach. Although within the larger regional assemblage, more males than females were identified (61% vs 39%), the site-specific analysis at Sand Canyon Pueblo suggests that adult male and female turkeys were present in a relatively even ratio.

Keyworks: Ancient DNA; Sex Identification; Sand Canyon Pueblo; Turkey (*Meleagris gallopavo*)

1. INTRODUCTION

In archaeological contexts, the ability to identify the sex of animal remains accurately is important for understanding past hunting practices (D'errico and Vanhaeren, 2002; Stiner, 1990; Weinstock, 2000), incipient animal domestication and herd management (Zeder and Hesse, 2000; Zeder, 2015), or ritual activities (Cultraro, 2004; Groot, 2008; Wilson, 1999). In the Southwest United States, several zooarchaeological studies have investigated the sex ratio exhibited in faunal assemblages to gain an understanding of how turkeys (*Meleagris gallopavo*) were managed and exploited in pre-contact

periods (Badenhorst, 2008; Badenhorst et al., 2012; McKusick, 1981; Munro, 1994). In addition to using discrete traits such as medullary bone (laying females) or tarsometatarsi spurs (males), osteometric sex identification techniques can be extremely accurate with sexually dimorphic animals like turkeys, with males being larger than the females (Badenhorst et al., 2012; Gilbert, 1985; Manin et al., this volume; McKusick, 1980; Pelham and Dickson, 1992). Although the greatest lengths of complete elements are typically analyzed (Munro, 1994; Senior and Pierce, 1989), epiphyseal dimensions of even fragmented long bones can produce distinctive sex groupings (Badenhorst et al., 2012; Speller, 2009). Depending on the element being studied, however, there can be considerable overlap in the measurement ranges for males and females. Additionally, osteometric approaches are most appropriate when conducted on large, regional assemblages that can ascertain the range of variation present in the population (Badenhorst et al., 2012; Breitburg, 1988; Davis, 1987; Fothergill 2013), and may be less reliable when only a few remains are present. In these cases, DNA-based sex identification techniques can be more accurate, especially when dealing with highly fragmented or juvenile archaeological bones (Svensson et al., 2008).

1.1 Challenges with existing DNA-based sex identification techniques

Previous ancient molecular approaches for sex identification have targeted amelogenin genes (Haak et al., 2008; Lassen et al., 2000; Shaw et al., 2015; Svensson et al., 2012), zinc finger protein genes (ZFX/Y)(Svensson et al., 2008) sex-linked Y- or W-chromosome sequences (Allentoft et al., 2010; Bunce et al., 2003; Cappellini et al., 2004) or a combination of markers (Arslan et al., 2011; Pagès et al., 2009; Schmidt et al., 2003). In many cases, molecular sex identification techniques suffer from a high failure rate, due to the low quantity of single-copy sex markers in archaeological samples (Alonso et al., 2004; Pääbo et al., 2004). Although whole genome approaches are proving successful to identify the sex of ancient humans (Skoglund et al., 2013), the economic cost and bioinformatic challenges for population level characterization of faunal populations remain considerable.

Targeting highly repetitive elements within the nuclear genome is one cost-effective method to increase the success rate of DNA amplification. Most eukaryotic genomes contain significant amounts of highly repetitive DNA (hrDNA) sequences (Charlesworth et al., 1994), especially on the non-recombining regions of sex chromosomes. In birds, some of these repetitive regions are located on the female-specific W-chromosome genes (Saitoh et al., 1989; Tone et al., 1982). With repetitive

copy numbers of approximately 10,000 (Saitoh et al., 1989), W-chromosome hrDNA sequences provide the potential for increasing the sensitivity of sex identification techniques for archaeological bird bones.

Here, we propose a new, highly-sensitive sex identification technique for ancient turkey remains based on the co-amplification of sex-chromosome hrDNA and mitochondrial DNA (mtDNA) loci. The amplification of hrDNA maximizes the potential for nuclear DNA amplification, while the mtDNA acts as an internal positive control to ensure correct PCR set-up, to monitor for contamination, and to estimate DNA preservation. The hrDNA can identify the heteogametic sex (in this case female turkeys), and sex can be rapidly assessed based on a visual analysis of elecrophoresis gel. To test the accuracy and sensitivity of this technique, we applied it to 20 modern turkey samples and 117 archaeological turkey bones which had demonstrated adequate mtDNA preservation (Speller, 2009; Speller et al., 2010). The archaeological samples were selected from various sites and time periods to test the overall efficacy of the method at a regional level, as well as site-specific component examining one particular archaeological site (Sand Canyon Pueblo) where turkeys were heavily exploited.

1.2 Sand Canyon Pueblo

The site of Sand Canyon Pueblo is located in the center of the Northern San Juan Region, within the Colorado Plateau. Occupied from the 1240s until the last years of the 13th century, this Pueblo III site was a large aggregated community (ca. 420 rooms, 90 kivas, 14 towers, an enclosed kiva, and a D-shaped bi-wall structure), which would have been occupied by around 400-600 individuals (Kuckelman, 2007).

The faunal assemblages from Sand Canyon Pueblo and other nearby sites have been extensively analyzed (Driver et al., 1999; Muir and Driver, 2002; Munro, 1994). Muir's (2007, 1999) study of the Sand Canyon faunal assemblage indicated that approximately two-thirds of the assemblage was composed of mammals (mostly lagomorphs, small rodents and artiodactyls), one third composed of birds (mainly turkey, or "large birds"), as well as a few less common taxa of amphibian, reptile, and gastropods (Muir 1999:46). Galliformes dominated the midden and outdoor deposits throughout the site and the distribution and quantity of Galliformes (and lagomorphs) within domestic refuse deposits suggests their value as a commonplace food item. Osteometric analysis of more complete elements

suggested that adult male and female turkeys were raised in relatively equal proportions at the site (Badenhorst et al., 2012). Our current study explores to what extent this pattern is evident in highly fragmented remains (mostly humeral shafts) using the molecular mtW approach. The turkey samples from the site provide an excellent opportunity to increase the representativeness of the assemblage (by including fragmentary remains) to obtain accurate sex ratio for a more complete understanding of human-turkey interactions.

2. MATERIAL AND METHODS

2.1 Archaeological turkey bones

One hundred and seventeen archaeological turkey bones were obtained from 25 sites archaeological sites in the Southwest United States dating from 700-1700AD (Speller *et al.* 2010) (Table S1). Humeri were preferentially selected since significant size differences exist between male and female turkey humeri, with little to no overlap, between the sexes, in the measurements of humeral greatest length (GL), proximal breadth (Bp) and distal breadth (Bd) (Gilbert, 1985; McKusick, 1986). Focusing on a single element would also reduce the likelihood of sampling the same individual. Samples were photographed, measured and compared to osteological criteria to ensure they were consistent with *M. gallopavo* (Gilbert et al., 1996; Von den Driesch, 1976) (Table S1).

The 32 turkey remains from Sand Canyon Pueblo were recovered from 13 different contexts within four structures which included two D-shaped tower blocks (Blocks 200 and 1000), one typical residential unit block (block 1200), and one “public architectural block”, the D-shaped block (1500) (Table S2).

2.2 DNA extraction and amplification

DNA was extracted from the archaeological turkey bone samples in the dedicated ancient DNA laboratory in the Department of Archaeology at Simon Fraser University, using a modified silica spin protocol (Speller et al., 2010; Yang et al., 1998). Comprehensive contamination controls were followed throughout the analyses, including (i) the use of a dedicated ancient DNA facilities; (ii) a vigorous decontamination protocol of the bone samples prior to DNA extraction; (iii) the inclusion of multiple blank extracts and PCR negative controls (Speller et al., 2010).

Primers were designed to target a hrDNA fragment of the turkey W-chromosome. Saitoh et al.'s (1989) study of highly repetitive regions of the turkey W-chromosome indicated that a 400bp PstI unit repeated approximately 10,000 times within the diploid genome of the female turkey, and was absent within the male turkey genome. Primers TK-F176-W and TK-R320-W were designed to target a 144bp fragment this female-specific bent-repetitive DNA sequence (Table 1). These PstI primers were included in a co-amplification reaction with previously published primers TK-F315/TK-R567 (Speller et al., 2010) designed to amplify a 222bp fragment of the turkey mtDNA D-loop. The co-amplification of the W-chromosome and mtDNA (mtW co-amp) was designed to preferentially amplify the W-chromosome: 1) the W-chromosome fragment was designed to be shorter than the D-loop fragment; and 2) the primers were added to the co-amplification reaction in a ratio of 10:1. This primer ratio allowed for the W-chromosome to be preferentially amplified when present, but still contained adequate amounts of D-loop primer to act as an internal positive control.

PCR co-amplifications were conducted in a Mastercycler® ep (Eppendorf, Hamburg, Germany) in a 30 µL reaction volume containing 50 mM KCl and 10 mM Tris-HCl, 2.5 mM MgCl2, 0.2 mM dNTP, 1.5 mg/mL BSA, 0.6µM each W-chromosome primer, 0.06µM each D-loop primer, 3 µL DNA sample and 2.25-3.75 U AmpliTaq Gold™ LD (Life Technologies Corporation, Carlsbad, CA). Five µL of PCR product were separated by electrophoresis on 2% agarose gel and visualized using SYBR Green® (Life Technologies Corporation, Carlsbad, CA) on a Dark Reader (Clare Chemical Research, Inc, Dolores, CO).

Sex identities were assigned to individual samples based on visual analysis of the electrophoresis gel results: the amplification of the W-chromosome fragment (with or without a mtDNA amplification) indicated a female bird; the sole amplification of the mtDNA fragment indicated a male bird; failed amplification of both fragments indicated a PCR amplification failure (Figure 1). Samples identified as male in initial reactions were re-amplified to ensure that W-chromosome amplification failure was not responsible for a false positive result. The genetic sex identifications obtained through the mtW co-amp were compared to the morphological size of the bone elements to test the reliability of the genetic sex identification for the 33 complete humeri from mature individuals.

2.3 Modern turkeys

Modern turkey samples were also analyzed to help validate the results of mtW co-amp technique from individuals of known sex. Twenty turkey phalanges were collected from recently slaughtered birds at Valley Creek Farm, Victoria, BC (Table 2); the birds were identified as males or females based on morphological traits, such as snood size and beard length. Approximately 20mg of soft tissue was extracted using the Qiagen DNeasy Blood and Tissue kit following the manufacturer's protocol.

First, the modern turkey samples underwent genetic sex identification using the mtW co-amp technique with PstI and mtDNA primers at a ratio of 10:1. PCR amplifications were conducted for 30 cycles in a Mastercycler® ep (Eppendorf, Hamburg, Germany) in a 25 µL reaction volume containing 50 mM KCl and 10 mM Tris-HCl, 2.5 mM $MgCl_2$, 0.2 mM dNTP, 1.5 mg/mL BSA, 0.6µM each W-chromosome primer, 0.06µM each D-loop primer, 1.0-1.5 µL DNA sample and 1.25 U AmpliTaq Gold™ LD (Life Technologies Corporation, Carlsbad, CA). Sex identities were assigned based on visual analysis of the electrophoresis gels as described above.

Next, the modern samples were also tested with a primer set designed to target single copy W-chromosome HINT gene sequences to confirm the sex identities. Primers TKW-F268 and TKW-R482 were designed to amplify 196bp of the female-specific HINTW gene (based on GenBank isolate AY713488) (Backström et al., 2005) (Table 1). These primers were included in simplex PCR reactions with the same conditions as listed above. Sex identifications were again conducted via electrophoresis gel: the amplification for the HINTW fragment indicates female birds, while amplification failure indicates male birds. Three successfully amplified samples were randomly selected for sequencing using forward and reverse primers to ensure that the targeted HINTW gene fragment was being amplified.

3. RESULTS

3.1 Sex identification of modern samples

All 20 modern turkeys produced positive amplifications with the mtW co-amp technique (Figure 2), and in all but one case, the morphological and genetic sex identifications yielded consistent results. Sample MTU18 was morphologically identified as female, although both the mtW co-amp and HINTW tests indicated a male individual. This discrepancy is likely due to a morphological misidentification as secondary sexual characteristics, such as snood development can vary between individuals,

especially when relatively immature individuals are being examined (as is the case with the vast majority of modern turkeys slaughtered for meat). These mtW co-amp sex identifications were confirmed using the HINTW primer set in all cases (Table 2).

3.2 Sex identification of ancient turkey samples

PCR amplifications were obtained for 101 of 117 archaeological bone samples, an 86% success rate for DNA recovery. Results of the initial and repeat sex identification tests are found in Tables S1& S2. Seventy-five of the 85 archaeological bones from the regional assemblage were successfully identified, 29 of which were female (39%) and 46 male (61%). At the site of Sand Canyon Pueblo, 12 samples were identified as female, 14 as male, and 6 were unidentified. Using the same primer ratio of 1:10 of D-loop to PstI, a relatively even co-amplification was achieved for most female samples (Figure 3).

3.3 Correlation between genetic sex and morphological size

The obtained genetic sex was compared to the morphological size of the archaeological bone samples using measurements of complete humeri (GL, Bp and Bd). Thirty-one of the 33 complete mature humeri were identified using the mtW co-amp technique, resulting in 19 females and 12 males. The genetic sex identified using the mtW co-amp technique corresponded with the morphological size for all 31 of the successfully identified samples, with no overlap between the measurements of the largest females and the smallest males (Figure 4). The two humeri which failed the mtW co-amp were female, based on morphological size.

4. DISCUSSION

4.1 Efficacy of the mtW technique

The accuracy and effectiveness of the mtW co-amp technique are demonstrated through a number of observations. First, the mtW co-amp technique confirmed the sex identity for 19 of the 20 modern birds. Though the mtW co-amp results failed to match the morphological sex for one of the birds (MTU18), further genetic testing indicated that the initial morphological identification was likely incorrect.

In 100% of cases where the mtW co-amp was applied to complete archaeological humeri (n=31), the mtW co-amp sex identity matched the predicted sex based on element size. This result indicates that the co-amplification primer ratio was effective in amplifying both the W-chromosome and mtDNA fragment when they were present in sufficient quantities, but suitably balanced to avoid the preferential amplification of mtDNA alone.

The key to the success of the mtW co-amp lies in the design of optimal primers and PCR conditions. Ideally, the hrDNA sex-chromosome fragment should be shorter than the mtDNA fragment in order to preferentially amplify the nuclear DNA and avoid false-positive results due to allele drop-out. This is particularly critical in ancient samples, where the target fragments may be differentially preserved. Balanced co-amplification of the two fragments can be achieved by adjusting primer ratios, while uneven amplification may be indicative of differential nuclear or mtDNA preservation. For example, although both DNA markers were successfully amplified in modern and ancient females, a comparison of Figure 2 and 3 shows that the same primer ratios (1:10) usually result in relatively weaker PstI amplification in modern females, but slightly stronger PstI amplifications in some ancient turkey female samples (demonstrated in TU1091 of Figure 3).

The majority of DNA sex identification techniques interpret the absence of PCR amplification for a particular sex chromosome to reflect the biological absence of that chromosome. However, failed PCR amplification in ancient samples can also be the result of PCR inhibition, insufficient DNA templates and amplification competition with other primers/markers. Our proposed mtW co-amp method is capable of detecting these factors: as an internal control, the mtDNA marker can serve as an indication PCR inhibition. Primer ratios can also be modified to consistently preferentially amplify the W-fragment; once optimized, the absence of the W-fragment (but the presence of mtDNA) can reliably point to the male identity of the sample.

In female birds, the successful amplification of the mtDNA fragment, and failure of the W-chromosome fragment would lead to a false-positive male identification. Though a greater proportion of male turkeys was identified in the ancient remains, the correspondence between the morphological and genetic sex identities among complete humeri suggests that this biased sex ratio represents an accurate reflection of the proportion of male and female turkeys in the larger regional assemblage.

In addition to acting as an internal positive control, the amplification of the mtDNA fragment offers other advantages. First, the co-amplification technique may also detect possible contamination if sequencing of the hrDNA and mtDNA fragments indicates two different species. Depending on the region that is targeted, the mtDNA can also be used to confirm species, and identify subspecies or geographic variants (Pagès et al., 2009), as well as providing a reproducibility test for mtDNA fragments amplified in a simplex reaction. Moreover, the co-amplification of sex-chromosome and mtDNA acts to conserve ancient DNA template, and reduce the overall costs and time associated with the analysis of ancient remains.

4.2 Archaeological turkey sex ratios

Within the regional study, the DNA analysis indicated a higher frequency of adult males than females. Significant differences in the exploitation of one sex over the other through time may point to flock management practices designed for particular products, e.g. feathers or meat. However, samples in this study were selected from many sites over a 1000 year time period, and thus are not likely to be representative of flock management practices at the site level. The larger number of males, however, may reflect differential preservation of male and female bones or sampling bias.

Bone mineral density plays a role in the survivorship of turkey skeletal elements and portions, with denser and larger bones possessing a higher potential for survival (Dirrigl, 2001; Grayson, 1979). Since turkeys are sexually dimorphic, male turkey bones are generally larger and more robust than female bones, therefore possessing a greater potential for survival in the archaeological record. Furthermore, the completeness of an element, and the retention of key diagnostic features, will affect its identifiability to the species level (Dirrigl, 2001). Therefore, if less fragmented elements are preferentially selected for analysis, then taphonomic and sampling bias may contribute to higher percentage of identified males. Molecular sex identification techniques, which can be applied to even highly fragmented collections, may be useful for uncovering such biases in osteometric analyses of more complete elements.

4.3 Sand Canyon Pueblo

At the site of Sand Canyon Pueblo, female and male turkeys are present in nearly equal numbers (46% vs 54% of identified samples, respectively). This sex ratio is very similar to Munro's (1994, p.

77) osteological analysis of tibiotarsi in the Sand Canyon locality, where she found that females and males represented 44% and 56% of the adult populations, respectively. Badenhorst's (2008:77) analysis of tarsometatarsi at the nearby Albert Porter Pueblo also found equal numbers of toms and hens, suggesting little or no differences in the treatments of sexes in terms of meat and/or secondary products.

It could be argued that demographic profiles of birds raised exclusively for meat will display a higher number of young adult males and older adult females, while flocks maintained for feathers, should display even numbers of male and female adult birds. If meat is the principal role of the animal, males should be culled as soon as they reach maturity, since it is most energy efficient to butcher an animal when it reaches adult size, rather than maintaining it after adulthood when its overall growth slows dramatically (Greenfield et al., 1988). Females, on the other hand, may be maintained as breeding stock, or potentially for their eggs, which could be used for food, glue or paint ingredients (McKusick, 1986). Maintaining a stock for their feathers should demonstrate a more even sex ratio of young and old adults, since the birds of both sexes produce feathers; archaeologists have posited that access to feathers may have been one of the initial motivations for turkey domestication in the American Southwest (McKusick, 1980, 1986; Munro, 2011). Adult gobblers (both in the wild and domesticated forms) tend to produce more iridescent, lustrous, and richly coloured plumage than hens (Babcock, 1902; Ligon, 1946). Thus, an even sex ratio may suggest that adult males were maintained for their rich plumage, and adult females both for their feathers and as breeding stock. A mixed strategy is also possible, for examples birds sacrificed for their feathers may also have been eaten, while birds killed for food may also have been plucked for feathers. A combination of approaches, including the analysis of depositional contexts, butchery marks, age profiles, and sex ratios validated with molecular techniques, are essential for reconstructing the husbandry and exploitation patterns of domestic turkeys in archaeological sites.

5. CONCLUSION

Here, we present an accurate and sensitive molecular sex identification technique for ancient turkey remains. By targeting hrDNA, we demonstrate a high success rate for the nuclear DNA amplification. Although whole genome sequencing coupled with next generation sequencing is fast becoming the norm in ancient DNA studies (Allentoft et al., 2015; Hofreiter et al., 2015; Teasdale et al., 2015), this

co-amplification techniques provides a relatively rapid, and cost-effective approach, without the need for sequencing. While pronounced sexual dimorphism in turkeys means that the majority of long bones can be distinguished osteometrically, the mtW technique is useful for identifying juvenile bones, or other turkey remains such as coprolites and feathers. Accurate sex profiles will always be challenging when working with highly fragmentary remains, nevertheless, the mtW approach offers a cost-effective method for validating osteometric criteria for small (or even large) zooarchaeological assemblages.

This co-amplification technique can also be modified based on the sex chromosome mechanisms of various animals to identify the heterogametic sex; for example, targeting the W-chromosome hrDNA in birds and fish, and the Y-chromosome hrDNA in mammals. The feasibility of designing hrDNA primers is enhanced by the identification of sex-chromosome hrDNA in humans (Jin et al., 2012) and many non-human animal species (Kageyama et al. 2004; Gosálvez et al. 2010; McGraw et al. 1988; Appa Rao and Totey 1999; Saitoh et al. 1989). Thus, this versatile co-amplification approach offers a highly sensitive and accurate technique for the sex identification of archaeological remains, and other degraded or low template DNA samples, such as museum samples, evidentiary or non-invasive wildlife samples, and paleontological materials.

SUPPLEMENTARY DATA

Supplementary Table S1 Provenience, measurements and sex identification results for archaeological turkey humeri

Supplementary Table S2 Provenience, measurements and sex identification results for Sand Canyon archaeological turkey bones

ACKNOWLEDGEMENT

We thank Dr R. Garvin, Dr J. Kelley, Dr B. Vierra, Dr C. Cameron, as well as all the colleagues at the National Park Service, Western Archeological and Conservation Center, Crow Canyon Archaeological Center, Museum of Northern Arizona, Anasazi Heritage Center, and Arizona State Museum who helped us access archaeological bone samples for analysis. Thanks to Dr Jonathan Driver for assistance in initial research design and interpretation of results. Our thanks also to Andrew Barton, Dr Shaw Badenhorst and Dr Tyr Fothergill for their assistance in osteological analysis, and to Dr

Ursula Arndt, Krista McGrath, and Sarah Padilla for technical assistance in the Simon Fraser University ancient DNA laboratory, and for discussion on the DNA results. Our thanks to the two anonymous reviewers who provided helpful comments on the manuscript, and particularly to Reviewer 1 for conceptualizing Figure 1. Funding was provided through a Wenner-Gren Foundation Dissertation Fieldwork grant to C.F.S [Grant 7744] and through a Social Sciences and Humanities Research Council of Canada grant to D.Y.Y.

REFERENCES

Allentoft, M.E., Bunce, M., Scofield, R.P., Hale, M.L., Holdaway, R.N., 2010. Highly skewed sex ratios and biased fossil deposition of moa: ancient DNA provides new insight on New Zealand's extinct megafauna. Quat. Sci. Rev. 29, 753–762.

Allentoft, M.E., Sikora, M., Sjögren, K.-G., Rasmussen, S., Rasmussen, M., et al., 2015. Population genomics of Bronze Age Eurasia. Nature 522, 167–172.

Alonso, A., Martín, P., Albarrán, C., García, P., García, O., de Simón, L.F., García-Hirschfeld, J., Sancho, M., de La Rúa, C., Fernández-Piqueras, J., 2004. Real-time PCR designs to estimate nuclear and mitochondrial DNA copy number in forensic and ancient DNA studies. Forensic Sci. Int. 139, 141–149.

Appa Rao, K.B., Totey, S.M., 1999. Cloning and sequencing of buffalo male-specific repetitive DNA: sexing of in-vitro developed buffalo embryos using multiplex and nested polymerase chain reaction. Theriogenology 51, 785–797.

Arslan, S., Açıkkol, A., Korkmaz, E.M., 2011. The optimization of aDNA extraction protocol and sex determination of Bronze Age individuals from Oylum Höyük (Kilis, Turkey). Turk. J. Biol. 35, 647–653.

Babcock, H.S., 1902. The bronze turkey, in: Myrick, H. (Ed.), Turkeys and How to Grow Them. Orange Judd Company, New York, pp. 16–18.

Backström, N., Ceplitis, H., Berlin, S., Ellegren, H., 2005. Gene conversion drives the evolution of HINTW, an ampliconic gene on the female-specific avian W chromosome. Mol. Biol. Evol. 22, 1992–1999.

Badenhorst, S., 2008. The zooarchaeology of great house sites in the San Juan Basin of the American Southwest. Department of Archaeology. Simon Fraser University, Burnaby, BC.

Badenhorst, S., Lyle, R., Merewether, J., Driver, J., Ryan, S., 2012. The Potential of Osteometric Data for Comprehensive Studies of Turkey (Meleagris gallopavo) Husbandry in the American Southwest. Kiva 78, 61–78.

Breitburg, E., 1988. Prehistoric New World turkey domestication: origins, developments, and consequences (PhD Diss.). Southern Illinois University at Carbondale

Bunce, M., Worthy, T.H., Ford, T., Hoppitt, W., Willerslev, E., Drummond, A., Cooper, A., 2003. Extreme reversed sexual size dimorphism in the extinct New Zealand moa Dinornis. Nature 425, 172–175.

Cappellini, E., Chiarelli, B., Sineo, L., Casoli, A., Di Gioia, A., Vernesi, C., Biella, M.C., Caramelli, D., 2004. Biomolecular study of the human remains from tomb 5859 in the Etruscan necropolis of Monterozzi, Tarquinia (Viterbo, Italy). J. Archaeol. Sci. 31, 603–612.

Charlesworth, B., Sniegowski, P., Stephan, W., 1994. The evolutionary dynamics of repetitive DNA in eukaryotes. Nature 371, 215–220.

Cultraro, M., 2004. Food for the gods: animal consumption and ritual activities in the early Bronze Age Sicily. PECUS. Man and animal in antiquity 1–10.

Davis, S.J.M., 1987. The Archaeology of Animals. Haven and London.

Dirrigl, F J, 2001. Bone mineral density of wild turkey (Meleagris gallopavo) skeletal elements and its effect on differential survivorship. J. Archaeol. Sci. 28, 817–832.

Dirrigl, Frank J, 2001. Differential identifiability between chosen North American gallinaceous skeletons and the effect of differential survivorship. Acta Zool. Cracov. 45, 357–367.

Driver, J.C., Brand, M.J., Lester, L., Munro, N.D., 1999. Faunal Studies, in: Varien, M. (Ed.), The Sand Canyon Archaeological Project: Site Testing [HTML Title]. Chapter 18. Available: http://www.crowcanyon.org/sitetesting. Date of use: 20 April 2009.
D'errico, F., Vanhaeren, M., 2002. Criteria for Identifying Red Deer (Cervus elaphus) Age and Sex from Their Canines. Application to the Study of Upper Palaeolithic and Mesolithic Ornaments. J. Archaeol. Sci. 29, 211–232.
Fothergill, B.B.C., 2013. The bird of the next dawn: The husbandry, translocation and transformation of the turkey (PhD Diss) University of Leicester.
Gilbert, B.M., Martin, L.D., Savage, H.G., 1996. Avian osteology. Missouri Archaeological Society, Inc., Columbia.
Gilbert, M., 1985. Avian osteology.
Gosálvez, J., Crespo, F., Vega-Pla, J.L., López-Fernández, C., Cortés-Gutiérrez, E.I., Devila-Rodriguez, M.I., Mezzanotte, R., 2010. Shared Y chromosome repetitive DNA sequences in stallion and donkey as visualized using whole-genomic comparative hybridization. Eur. J. Histochem. 54, e2.
Grayson, D.K., 1979. On the quantification of vertebrate archaeofaunas, in: Schiffer, M.B. (Ed.), Advances of Archaeological Method and Theory. Academic Press, Inc., New York, NY, pp. 199–237.
Greenfield, H.J., Chapman, J., Clason, A.T., Gilbert, A.S., Hesse, B., Milisauskas, S., 1988. The origins of milk and wool production in the Old World: a zooarchaeological perspective from the Central Balkans [and Comments]. Curr. Anthropol. 29, 573–593.
Groot, M., 2008. Animals in ritual and economy in a Roman frontier community: Excavations in Tiel-Passewaaij. Amsterdam University Press.
Haak, W., Brandt, G., de Jong, H.N., Meyer, C., Ganslmeier, R., Heyd, V., Hawkesworth, C., Pike, A.W.G., Meller, H., Alt, K.W., 2008. Ancient DNA, Strontium isotopes, and osteological analyses shed light on social and kinship organization of the Later Stone Age. Proc. Natl. Acad. Sci. U. S. A. 105, 18226–18231.
Hofreiter, M., Paijmans, J.L.A., Goodchild, H., Speller, C.F., Barlow, A., Fortes, G.G., Thomas, J.A., Ludwig, A., Collins, M.J., 2015. The future of ancient DNA: Technical advances and conceptual shifts. Bioessays 37, 284–293.
Jin, S., Lin, X.M., Law, H., Kwek, K.Y.C., Yeo, G.S.H., Ding, C., 2012. Further improvement in quantifying male fetal DNA in maternal plasma. Clin. Chem. 58, 465–468.
Kageyama, S., Yoshida, I., Kawakura, K., Chikuni, K., 2004. A novel repeated sequence located on the bovine Y chromosome: its application to rapid and precise embryo sexing by PCR. J. Vet. Med. Sci. 66, 509–514.
Kuckelman, K., 2007. The archaeology of Sand Canyon Pueblo: Intensive excavations at a late-thirteenth-century village in southwestern Colorado [HTML Title]. Available: http://www.crowcanyon.org/sandcanyon. Date of use: 20 April 2009.
Lassen, C., Hummel, S., Herrmann, B., 2000. Molecular sex identification of stillborn and neonate individuals (" Traufkinder") from the burial site Aegerten. Anthropol. Anz. 1–8.
Ligon, J.D., 1946. History and management of Merriam's wild turkey. The University of New Mexico Press, Albuquerque.
Manin, A., Cornette, R., Lefèvre, C., Sexual dimorphism among Mesoamerican turkeys: a key for understanding past husbandry, Journal of Archaeological Science: Reports (this volume)
McGraw, R.A., Jacobson, R.J., Akamatsu, M., 1988. A male-specific repeated DNA sequence in the domestic pig. Nucleic Acids Res. 16, 10389.
McKusick, C.R., 1986. Southwest Indian turkey: Prehistory and comparative osteology. Southwest Bird Laboratory, Globe, AZ.
McKusick, C.R., 1981. The faunal remains of Las Humanas, in: Hayes, A.C. (Ed.), Contributions to Gran Quivira Archaeology, Gran Quivira National Monument, New Mexico, Publications in Archeology. National Park Service, U.S. Dept. of Interior, Washington, pp. 39–65.
McKusick, C.R., 1980. Three groups of turkeys from Southwestern archaeological sites. Contrib. Sci. Nat. Hist. Mus. Los Angeles Co. 330:, 225–235.
Muir, R.J., 2007. Faunal remains, in: Kuckelman, K. (Ed.), The Archaeology of Sand Canyon Pueblo: Intensive Excavations at a Late-Thirteenth-Century Village in Southwestern Colorado [HTML Title]. Available: http://www.crowcanyon.org/sandcanyon. Date of use: 20 April 2009.
Muir, R.J., 1999. Zooarchaeology of Sand Canyon Pueblo, Colorado. Department of Archaeology. Simon Fraser University, Burnaby, BC.
Muir, R.J., Driver, J.C., 2002. Scale of analysis and zooarchaeological interpretation: Pueblo III faunal variation in the northern San Juan region. Journal of Anthropological Archaeology 21, 165–199.

Munro, N.D., 1994. An investigation of Anasazi turkey production in southwestern Colorado. Department of Archaeology. Simon Fraser University, Burnaby, BC.
Munro, N.D., 2006. The role of turkey in the Southwest, in: Sturtevant, W.C. (Ed.), Handbook of North American Indians, Vol. 3, Environment, Origins and Populations. Smithsonian Institution, Washington, pp. 463–469.
Pagès, M., Maudet, C., Bellemain, E., Taberlet, P., Hughes, S., Hänni, C., 2009. A system for sex determination from degraded DNA: a useful tool for palaeogenetics and conservation genetics of ursids. Conserv. Genet. 10, 897–907.
Pelham, P.H., Dickson, J.G., 1992. Physical characteristics, in: Dickson, J.G. (Ed.), The Wild Turkey: Biology and Management. Stackpole Books, Harrisburg, PA, pp. 32–45.
Pääbo, S., Poinar, H., Serre, D., Jaenicke-Despres, V., Hebler, J., Rohland, N., Kuch, M., Krause, J., Vigilant, L., Hofreiter, M., 2004. Genetic analyses from ancient DNA. Annu. Rev. Genet. 38, 645–679.
Saitoh, H., Harata, M., Mizuno, S., 1989. Presence of female-specific bent-repetitive DNA sequences in the genomes of turkey and pheasant and their interactions with W-protein of chicken. Chromosoma 98, 250–258.
Saitoh, Y., Saitoh, H., Ohtomo, K., Mizuno, S., 1991. Occupancy of the majority of DNA in the chicken W chromosome by bent-repetitive sequences. Chromosoma 101, 32–40.
Schmidt, D., Hummel, S., Herrmann, B., 2003. Brief communication: Multiplex X/Y-PCR improves sex identification in aDNA analysis. Am. J. Phys. Anthropol. 121, 337–341.
Senior, L.M., Pierce, L.J., 1989. Turkeys and domestications in the Southwest: Implications from Homol'ovi III. Kiva 54, 245–259.
Shaw, K.J., Brown, K.A., Brown, T.A., Haswell, S.J., 2015. Sex identification of ancient DNA samples using a microfluidic device. Methods Mol. Biol. 1274, 93–98.
Skoglund, P., Storå, J., Götherström, A., Jakobsson, M., 2013. Accurate sex identification of ancient human remains using DNA shotgun sequencing. J. Archaeol. Sci. 40, 4477–4482.
Speller, C.F., 2009. Investigating turkey (Meleagris gallopavo) domestication in the Southwest United States through ancient DNA analysis (PhD diss.). Department of Archaeology-Simon Fraser University.
Speller, C.F., Kemp, B.M., Wyatt, S.D., Monroe, C., Lipe, W.D., Arndt, U.M., Yang, D.Y., 2010. Ancient mitochondrial DNA analysis reveals complexity of indigenous North American turkey domestication. Proceedings of the National Academy of Sciences 107, 2807–2812.
Stiner, M.C., 1990. The use of mortality patterns in archaeological studies of hominid predatory adaptations. Journal of Anthropological Archaeology 9, 305–351.
Svensson, E.M., Götherström, A., Vretemark, M., 2008. A DNA test for sex identification in cattle confirms osteometric results. J. Archaeol. Sci. 35, 942–946.
Svensson, E.M., Telldahl, Y., Sjöling, E., Sundkvist, A., Hulth, H., Sjøvold, T., Götherström, A., 2012. Coat colour and sex identification in horses from Iron Age Sweden. Ann. Anat. 194, 82–87.
Teasdale, M.D., van Doorn, N.L., Fiddyment, S., Webb, C.C., O'Connor, T., Hofreiter, M., Collins, M.J., Bradley, D.G., 2015. Paging through history: parchment as a reservoir of ancient DNA for next generation sequencing. Philos. Trans. R. Soc. Lond. B Biol. Sci. 370. doi:10.1098/rstb.2013.0379
Tone, M., Nakano, N., Takao, E., Narisawa, S., Mizuno, S., 1982. Demonstration of W chromosome-specific repetitive DNA sequences in the domestic fowl, Gallus g. domesticus. Chromosoma 86, 551–569.
Von den Driesch, A., 1976. A guide to the measurement of animal bones from archaeological sites. Bull. Peabody Mus. Nat. Hist. Yale Univ. 1, 1–137.
Weinstock, J., 2000. Osteometry as a Source of Refined Demographic Information: Sex-Ratios of Reindeer, Hunting Strategies, and Herd Control in the Late Glacial site of Stellmoor, Northern Germany. J. Archaeol. Sci. 27, 1187–1195.
Wilson, B., 1999. Displayed or Concealed? Cross Cultural Evidence for Symbolic and Ritual Activity Depositing Iron Age Animal Bones. Oxford Journal of Archaeology 18, 297–305.
Yang, D.Y., Eng, B., Waye, J.S., Dudar, J.C., Saunders, S.R., 1998. Technical note: improved DNA extraction from ancient bones using silica-based spin columns. Am. J. Phys. Anthropol. 105, 539–543.
Zeder, M.A., 2015. Core questions in domestication research. Proceedings of the National Academy of Sciences. doi:10.1073/pnas.1501711112
Zeder, M.A., Hesse, B., 2000. The Initial Domestication of Goats (Capra hircus) in the Zagros Mountains 10,000 Years Ago. Science 287, 2254–2257.

TABLE AND FIGURES LEGENDS

Table 1 PstI and HINTW PCR primers used to amplify turkey W-chromosome fragments

Locus	Primer Name	Position*	Sequence (5' to 3')
PstI	TK-F176-W	176-198	CCAGAAATACCAATTATCTCCGC
	TK-R320-W	298-319	CGATAAAACTGGCATTTCCTGG
HINTW	TKW-F286	286-304	AAGCGATGCTCATTTCTGG
	TKW-R482	414-431	TCC GAC CTG CTC AAA ACC

Note: F and R in the primer name denotes forward and reverse primers, respectively. *Position number for PstI primer is based on *Meleagris gallopavo* GenBank isolate X17583 for female-specific 0.4 kb PstI repetitive unit. Position number for HINTW primers based on GenBank isolate AY713488.

Table 2 Sex identification results for modern turkey samples

Lab ID	Morphological Sex	mtW co-amp Sex ID	HINTW Sex ID	Final Sex ID
MTU10	Male	Male	Male	Male
MTU11	Male	Male	Male	Male
MTU12	Male	Male	Male	Male
MTU13	Male	Male	Male	Male
MTU14	Male	Male	Male	Male
MTU15	Female	Female	Female	Female
MTU16	Female	Female	Female	Female
MTU17	Female	Female	Female	Female
MTU18	Female	Male	Male	Male
MTU19	Female	Female	Female	Female
MTU20	Female	Female	Female	Female
MTU21	Female	Female	Female	Female
MTU22	Female	Female	Female	Female
MTU23	Female	Female	Female	Female

MTU24	Female	Female	Female	Female
MTU25	Male	Male	Male	Male
MTU26	Male	Male	Male	Male
MTU27	Male	Male	Male	Male
MTU28	Male	Male	Male	Male
MTU29	Male	Male	Male	Male

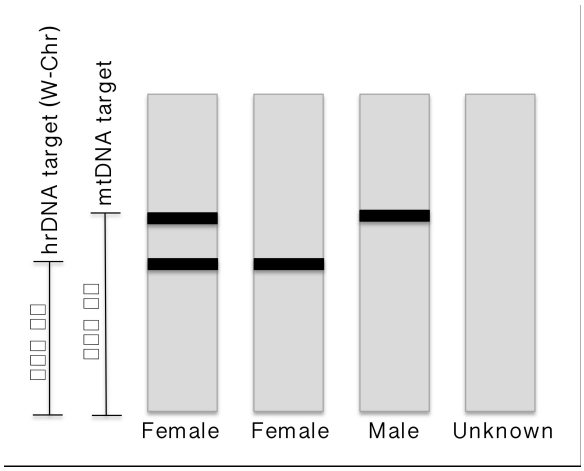

Figure 1 Conceptual diagram of the mtW co-amp approach. Sex identities can be assigned based on the visual analysis of electrophoresis gel: the amplification of the hrDNA fragment (with or without a mtDNA amplification) indicates a female bird; the sole amplification of the mtDNA fragment indicates a male bird; failed amplification of both fragments results in an unknown sex identification.

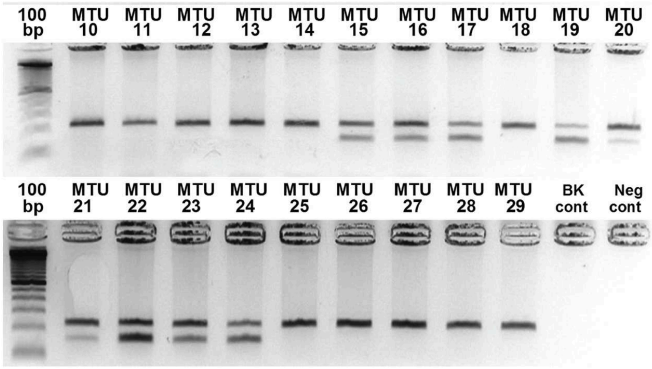

Figure 2 Electrophoresis gels displaying the mtW co-amp amplification results for the modern turkey samples (upper bands represent mtDNA D-loop fragments, while lower bands represent hrDNA (W PstI) fragments); 'BK cont' and 'Neg cont' indicate the blank extract and negative control, 100bp indicates 100 base pair ladder (Life Technologies Corporation, Carlsbad, CA).

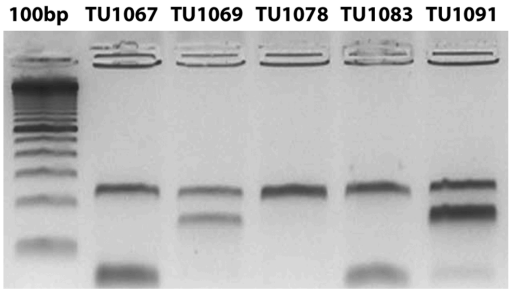

Figure 3 Electrophoresis gel showing the results of mtW co-amp. Upper bands represent mtDNA D-loop fragments, while lower bands represent hrDNA (W PstI) fragments); the short bands at the very bottom of the gel are primer-dimers; BK and Neg indicate the blank extract and negative control, 100bp indicates the 100 base pair ladder (Life Technologies Corporation, Carlsbad, CA)

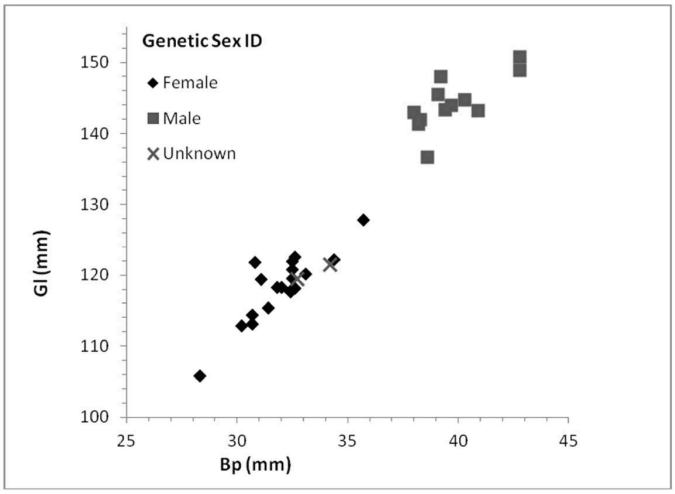

Figure 4 Scatterplot displaying the correspondence between genetic sex and morphological size for complete mature turkey humeri n=33 (Bp =maximal distal breadth, Gl=greatest length).

Table S1 Provenience, measurements and sex identification results for archaeological turkey humeri

Lab ID	Archaeological Site Name	Date (AD)	Portion	GL (mm)	Bp (mm)	Bd (mm)	Initial Sex ID	Repeat Sex ID	Final Sex ID
TU1	Hedley Ruin	1000-1300	Complete	119.4	31.1	24.4	Female	Female	Female
TU2	Hedley Ruin	1000-1300	Complete	142.0	38.3	29.0	Male	Male	Male
TU4	Hedley Ruin	1000-1300	Complete	118.3	31.8	25.0	Female	Female	Female
TU6	Hedley Ruin	1000-1300	Complete	117.8	32.4	25.5	Female	Female	Female
TU8	Comb Wash	1150-1250	Complete	121.8	30.8	24.5	Female	Female	Female
TU28	Mockingbird Mesa/CANM	900–1350	Complete	119.5	32.7	26.1	NA	-	NA
TU34	Stanton's Site	1230-1270	Complete	119.5	32.5	25.4	Female	Female	Female
TU88	Los Alamos	1275-1325	Complete	143.2	40.9	30.7	Male	Male	Male
TU90	Los Alamos	1275-1325	Complete	118.2	32.6	25.9	Female	-	Female
TU92	Los Alamos	1275-1325	Complete	112.9	30.2	23.9	Female	-	Female
TU97	Shields Pueblo	1150-1250	Complete	121.6	34.2	26.6	NA	-	NA
TU123	Bluff Great House	1150-1300	Complete	144.0	39.7	30.0	Male	Male	Male
TU124	Bluff Great House	1150-1300	Complete	144.7	40.3	29.6	Male	Male	Male
TU125	Bluff Great House	1150-1300	Complete	122.2	34.4	26.5	Female	Female	Female
TU126	Bluff Great House	1150-1300	Complete	122.5	32.6	25.0	Female	-	Female
TU1020	Tsa-ta'a, Canyon de Chelly	700-1300	Complete	117.6	32.4	25.3	Female	-	Female
TU1022	Tsa-ta'a, Canyon de Chelly	700-1300	Complete	113.1	30.7	23.8	Female	-	Female
TU1049	Gran Quivira	1300-1672	Complete	105.8	28.3	22.0	Female	-	Female
TU1053	Gran Quivira	1300-1672	Complete	136.6	38.6	29.3	Male	Male	Male
TU1054	Gran Quivira	1300-1672	Complete	115.4	31.4	25.2	Female	Female	Female
TU1067	El Morro	1280-1380	Complete	145.5	39.1	30.4	Male	Male	Male
TU1069	El Morro	1280-1380	Complete	118.3	32.0	25.5	Female	-	Female
TU1070	El Morro	1280-1380	Complete	143.3	39.4	30.6	Male	Male	Male
TU1078	Keet Seel	1250-1300	Complete	143.0	38.0	29.4	Male	Male	Male

TU1083	Keet Seel	1250-1300	Complete	141.3	38.2	29.4	Male	Male	Male
TU1091	Aztec Ruin	1105-1300	Complete	120.8	32.5	24.8	Female	-	Female
TU1093	Aztec Ruin	1105-1300	Complete	148.0	39.2	30.7	Male	Male	Male
TU1096	Aztec Ruin	1105-1300	Complete	114.3	30.7	24.2	Female	-	Female
TU1097	Aztec Ruin	1105-1300	Complete	121.9	32.5	25.4	Female	-	Female
TU1103	Point of Pines Pueblo	1200-1400	Complete	148.9	42.8	34.0	Male	Male	Male
TU1104	Point of Pines Pueblo	1200-1400	Complete	120.1	33.1	25.7	Female	-	Female
TU1105	Point of Pines Pueblo	1200-1400	Complete	150.7	42.8	33.5	Male?	Male	Male
TU1111	Grasshopper Pueblo	1300-1400	Complete	127.8	35.7	27.7	Female	Female	Female
TU13*	Aldea Sierritas	720-800	Complete/ Immature	137.4	32.0	26.4	Male	Male	Male
TU14*	Aldea Sierritas	720-800	Complete/ Immature	103.8	25.5	21.2	Male	Male	Male
TU5	Hedley Ruin	1000-1300	Proximal	-	32.7	-	Female	Female	Female
TU7	Comb Wash	1150-1250	Proximal	-	40.0	-	Male	Male	Male
TU11	Comb Wash	1150-1250	Distal	-	-	23.8	NA	-	NA
TU15	LeMoc Shelter	720-900	Shaft	-	-	-	Male	Male	Male
TU20	McPhee Village	820-980	Proximal	-	40.9	-	Male	Male	Male
TU22	McPhee Village	820-980	Distal	-	-	-	Male	Male	Male
TU24	Ida Jean Site	1050-1150	Proximal	-	32.3	-	Female	Female	Female
TU25	Ida Jean Site	1050-1150	Proximal	-	38.6	-	Male	Male	Male
TU26	Escalante Pueblo	1075-1250	Distal	-	-	30.9	Male	Male	Male
TU27	Escalante Pueblo	1075-1250	Shaft	-	-	-	Male	Male	Male
TU29	Mockingbird Mesa/CANM	900–1350	Proximal	-	38.7	-	Male	Male	Male
TU30	Mockingbird Mesa/CANM	900–1350	Shaft	-	-	-	Male	Male	Male
TU31	Mockingbird Mesa/CANM	900–1350	Shaft	-	-	-	Female	-	Female
TU32	Mockingbird Mesa/CANM	900–1350	Proximal	-	38.0	-	Male	Male	Male
TU33	Mockingbird Mesa/CANM	900–1350	Proximal	-	31.8	-	NA	-	NA

TU35	Stanton's Site	1230-1270	Shaft	-	-	-	NA	-	NA
TU36	Stanton's Site	1230-1270	Shaft	-	-	-	Male	Male	Male
TU37	Stanton's Site	1230-1270	Distal	-	-	32.2	Male	Male	Male
TU38	Stanton's Site	1230-1270	Distal	-	-	32.2	Male	Male	Male
TU39	Castle Rock	1250-1300	Shaft	-	-	-	Female	-	Female
TU40	Castle Rock	1250-1300	Proximal	-	39.5	-	Male	Male	Male
TU41	Castle Rock	1250-1300	Shaft	-	-	-	Male	Male	Male
TU42	Castle Rock	1250-1300	Proximal	-	39.5	-	Male	Male	Male
TU43	Castle Rock	1250-1300	Shaft	-	-	-	NA	-	NA
TU44	Mockingbird Mesa/CANM	700-1100	Proximal	-	38.3	-	Male	NA	NA
TU53	Mockingbird Mesa/CANM	1150-1300	Proximal	-	40.5	-	Male	Male	Male
TU54	Mockingbird Mesa/CANM	1150-1300	Shaft	-	-	-	Female	-	Female
TU89	Los Alamos	1275-1325	Complete/ Damaged	-	37.8	29.6	Male	Male	Male
TU91	Los Alamos	1275-1325	Proximal	-	37.2	-	Male	Male	Male
TU101	Shields Pueblo	1150-1250	Proximal	-	40.8	-	Male	Male	Male
TU105	Shields Pueblo	1150-1250	Proximal	-	-	-	Female	-	Female
TU107*	Shields Pueblo	1020-1060	Complete/ Immature	34.9	8.0	6.6	Male	Male	Male
TU115	Shields Pueblo	1150-1250	Proximal	-	34.1	-	Female	Female	Female
TU116*	Shields Pueblo	1020-1060	Complete/ Immature	51.5	11.0	9.4	Male	Male	Male
TU1003	Alamo Canyon	1150-1180	Complete/ Damaged	117.7	-	27.2	Female	-	Female
TU1004	Rainbow House	1400-1600	Distal	-	-	30.1	Male	Male	Male
TU1026*	Tsa-ta'a, Canyon de Chelly	700-1300	Proximal	-	41.2	-	Male	Male	Male
TU1033	Antelope House	700-1300	Proximal	-	39.6	-	Male	Male	Male
TU1034	Antelope House	700-1300	Proximal	-	32.0	-	Female	-	Female
TU1041	Gran Quivira	1300-1672	Proximal	-	45.5	-	Male	Male	Male
TU1052*	Gran Quivira	1300-1672	Complete/ Immature	125.7	33.8	25.2	Male	Male	Male

TU1066	El Morro	1280-1380	Proximal	-	38.8	-	Male	Male	Male
TU1072*	El Morro	1280-1380	Proximal	-	31.5	-	Male	Male	Male
TU1098*	Aztec Ruin	1105-1300	Complete/ Immature	136.8	35.0	27.1	Male	Male	Male
TU1101	Point of Pines Pueblo	1200-1400	Proximal	-	44.7	-	Male	Male	Male
TU1102	Point of Pines Pueblo	1200-1400	Proximal	-	34.3	-	NA	-	NA
TU1106*	Grasshopper Pueblo	1300-1400	Proximal	-	33.4	-	Male	Male	Male
TU1108	Grasshopper Pueblo	1300-1400	Proximal	-	45.1	-	NA	-	NA
TU1112	Grasshopper Pueblo	1300-1400	Proximal	-	33.3	-	NA	-	NA

* Immature individuals, not included in comparisons of morphological size and genetic sex ID.

Table S2 Provenience, measurements and sex identification results for Sand Canyon archaeological turkey bones

Lab ID	Block	Context	Element	Portion	GL (mm)	Bp (mm)	Bd (mm)	Initial Sex ID	Repeat Sex ID	Final Sex ID
TU55	200	Other	Humerus	Shaft/ immature	-	-	-	Male	Male	Male
TU56	200	Other	Humerus	Shaft	-	-	-	Male	Male	Male
TU57	200	Other	Humerus	Shaft	-	-	-	Female	-	Female
TU58	200	Other	Humerus	Shaft	-	-	-	Male	Male	Male
TU59	200	Other	Humerus	Distal +shaft	-	-	30.9	NA	-	NA
TU60	200	Kiva	Cervical vert	Complete	-	-	-	NA	-	NA
TU61	200	Kiva	Tibiotarsus	Distal	-	-	20.4	NA	-	NA
TU62	200	Kiva	Tibiotarsus	Distal/ immature	-	-	19.1	NA	-	NA
TU63	200	Kiva	Tibiotarsus	Distal	-	-	17.8	Female	-	Female
TU64	1200	Kiva	Humerus	Distal	-	-	-	NA	-	NA
TU65	200	Kiva	Tibiotarsus	Distal	-	-	20.7	Male	Male	Male
TU66	1200	Midden	Humerus	Shaft	-	-	-	Male	Male	Male
TU67	1200	Midden	Humerus	Shaft	-	-	-	Male	Male	Male
TU68	1200	Midden	Tarsometatarsus	Complete/ immature	88.8	15.1	16.9	Female	Female	Female
TU69	1200	Midden	Humerus	Distal	-	-	25.1	Female	-	Female

TU70	1200	Midden	Humerus	Proximal	-	-	-	Male	Male	Male
TU71	1200	Midden	Humerus	Shaft	-	-	-	Male	Male	Male
TU72	1000	Kiva	Humerus	Shaft	-	-	-	Female	-	Female
TU73	1000	Other	Humerus	Shaft	-	-	-	Female?	Female	Female
TU74	1000	Other	Humerus	Shaft	-	-	-	Male	Male	Male
TU75	1500	Other	Humerus	Shaft	-	-	-	Female	-	Female
TU76	1500	Other	Humerus	Shaft	-	-	-	Female	Female	Female
TU77	1500	Other	Humerus	Complete	144.8	-	-	Male	Male	Male
TU78	1500	Other	Humerus	Distal	-	-	24.3	Female	-	Female
TU79	1500	Other	Humerus	Shaft	-	-	-	NA		NA
TU80	1500	Other	Humerus	Shaft	-	-	-	Male	Male	Male
TU81	1500	Other	Humerus	Distal	-	-	24.8	Female	-	Female
TU82	1500	Other	Humerus	Complete	120.3	-	-	Female	-	Female
TU83	1500	Other	Humerus	Shaft	-	-	-	Male	Male	Male
TU84	1500	Other	Humerus	Shaft	-	-	-	Male?	Male	Male
TU85	1500	Other	Humerus	Proximal	-	39.4	-	Male	Male	Male
TU86	1000	Kiva	Humerus	Shaft	-	-	-	Female	-	Female