

Ancient mitochondrial DNA analysis reveals complexity of indigenous North American turkey domestication

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Although the cultural and nutritive importance of the turkey (*Meleagris gallopavo*) to precontact Native Americans and contemporary people worldwide is clear, little is known about the domestication of this bird compared to other domesticates. Mitochondrial DNA analysis of 149 turkey bones and 29 coprolites from 38 archaeological sites (200 BC–AD 1800) reveals a unique domesticated breed in the precontact Southwestern United States. Phylogeographic analyses indicate that this domestic breed originated from outside the region, but rules out the South Mexican domestic turkey (*Meleagris gallopavo gallopavo*) as a progenitor. A strong genetic bottleneck within the Southwest turkeys also reflects intensive human selection and breeding. This study points to at least two occurrences of turkey domestication in precontact North America and illuminates the intensity and sophistication of New World animal breeding practices.

Meleagris gallopavo | Southwest US | Ancestral Puebloans | Mesoamerica

Animal domestication revolutionized the lives of past peoples, their relationship with the environment, and their technological and social development. To more fully understand these achievements in precontact native North American cultures, it would be remiss to overlook one of the continent's few animal domesticates: the turkey. For the ancient peoples of the American Southwest and Mesoamerica, it was not only an important source of dietary protein, but yielded byproducts such as feathers and bones having both ritual and practical uses (1, 2). Archaeological investigations into turkey domestication have proven inconclusive, especially regarding the geographic origin(s) of the domestic birds, the number of domestications, and how humans spread the domestic stocks (2).

In Mesoamerica, sporadic evidence of turkey use appears as early as 800–100 BC (2). Domestic turkey stocks were established by at least AD 180 within the Tehuacán valley (3), with the South Mexican turkey (*Meleagris gallopavo gallopavo*) as their assumed wild progenitor (4). The first concrete archaeological evidence for domestic stocks in the Southwest begins around the same time (ca. 200 BC–AD 500), although the wild progenitor of this bird has been long debated (5). Although previous hypotheses assumed the initial domestication took place in Mesoamerica with subsequent dispersal of domestic turkeys into the Southwest (following the general pattern of cultigens like maize, squash, and beans) (6), more recent studies have pointed to a separate center of turkey domestication either within the Southwest proper (involving the local Merriam's wild turkey, *Meleagris gallopavo merriami*) (7), or in an area to the east involving the Eastern wild turkey, *Meleagris gallopavo silvestris* (8).

In both Mesoamerica and the Southwest, the occurrence and importance of domestic turkey stocks increased through time until contact with the Spanish at the beginning of the 16th century (9). On the basis of historic accounts, however, it seems that of the two stocks, only the Mesoamerican turkey has survived

into the present day as domesticates. Mexican turkeys were transported to Europe in the early 16th century and spread quickly across the continent (4). Over the following centuries, several varieties of turkey were developed in Europe and later imported back to the U.S. Atlantic seaboard in the 18th century, where some became the forerunners to the commercially raised breeds known today (10). Within the Southwest, the rapid contraction of the area occupied by village farmers in the 13th through 15th centuries (11), the effects of disease and conflict associated with Spanish Colonialism after 1539, and the subsequent introduction of European domesticates such as sheep and chicken, all contributed to dramatic declines in local turkey husbandry during the 18th and 19th centuries (7). To date, no studies have compared the genetic diversity of modern (or archaeological) domestic turkeys to their wild counterparts, either to verify our historic understanding of Mesoamerican turkey domestication, or to identify the wild progenitors of the Southwest domestic turkey.

To determine the relationship between Southwest and Mesoamerican turkey stocks, and more fully understand the domestication history of this important bird, we targeted 438 base pairs (bp) of control-region mitochondrial DNA (mtDNA) from 200 turkey samples (*SI Text*), including 149 ancient turkey bones and 29 coprolites recovered from 38 archaeological sites in the Southwest USA (Fig. 1). The archaeological samples were extracted in dedicated ancient DNA laboratories following published protocols for analysis and authentication (12–14). As the wild South Mexican turkeys are now thought to be extinct, mtDNA variation was characterized for this subspecies using 10 historic wild specimens, which were morphologically identified and cataloged as *M. g. gallopavo* by the Division of Birds at the Smithsonian Institution. In addition, 12 samples from North American commercially raised turkeys were analyzed. Complete sequences (i.e., spanning the entire 438 bp) were obtained from all 12 modern samples, 126 of 149 archaeological bones, 17 of 29 coprolites, and 4 of 10 skin specimens of *M. g. gallopavo*. These 159 complete sequences were combined with 276 domestic and North American wild turkey sequences obtained from GenBank

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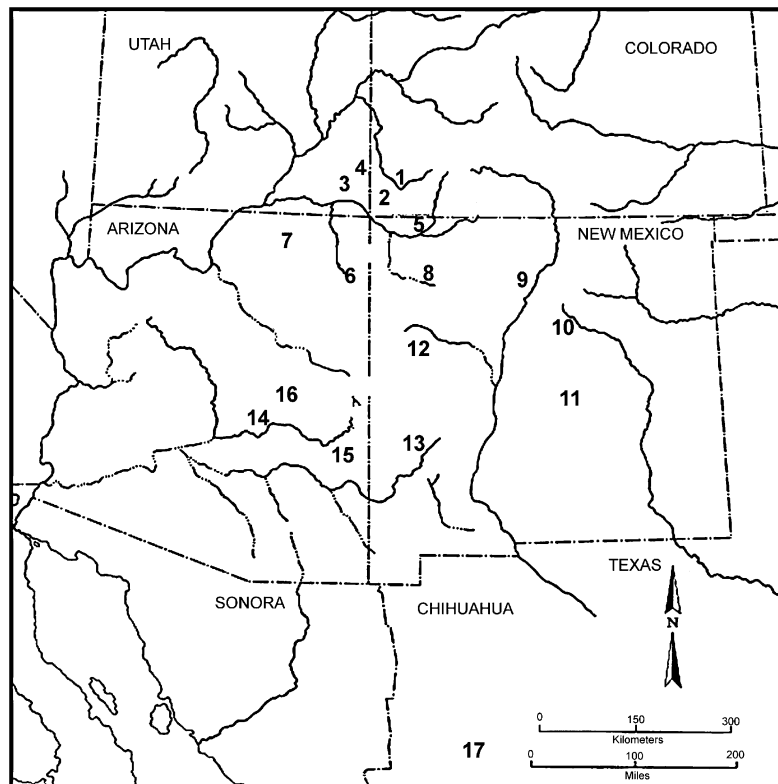


Fig. 1. Southwest archaeological sites from which turkey samples were obtained (1): Grass Mesa, LeMoc Shelter, Hamlet de la Olla, Aldea Sierritas, McPhee Village, Hanging Rock, House Creek Village, Escalante Pueblo; (2) Shields Pueblo, Mockingbird Mesa, Sand Canyon Pueblo, Stanton's Site, Castle Rock Pueblo, Ida Jean Site, Albert Porter; (3) Bluff Great House, Comb Wash, Turkey Pen; (4) Hedley Ruin; (5) Aztec Ruins; (6) Antelope House and Tsa-ta'a, Canyon de Chelly; (7) Keet Seel; (8) Pueblo Bonito; (9) Los Alamos, Alamo Canyon, Rainbow House; (10) South Pueblo and Forked Lightning (Pecos National Monument); (11) Gran Quivira; (12) El Morro; (13) Gila Cliff Dwellings; (14) Tonto National Monument; (15) Point of Pines; (16) Grasshopper Pueblo; (17) Calderón Site (Adapted with permission of the Crow Canyon Archaeological Center, 1999).

(see refs. 15, 16 for detailed geographic locations of sampled wild turkey populations). The sequences were compared using median joining networks (17) and phylogenetic trees (18, 19). In addition to the D-loop analysis, a 165-bp fragment of the *M. gallopavo* cytochrome *b* gene was also amplified and sequenced for a subset of the samples.

Results and Discussion

Twelve different mitochondrial haplotypes were observed within the sequenced samples (Table S1 and Fig. S1) falling roughly into 3 haplogroups (Fig. 2). The first haplogroup (H1) contained over 85% of the 143 successfully sequenced archaeological samples, and was dominated by a single haplotype (aHap1). Complete aHap1 sequences were recovered from 102 of the ancient bone samples and 14 of the ancient coprolite samples. The other four haplotypes were present in three (aHap1a) and one samples each (aHap1b, aHap1c, and aHap1d), all differing from aHap1 by single base pair mutations. The second haplogroup (H2), included 14.7% of the successfully sequenced archaeological samples. H2 contained six haplotypes, the most common of which, aHap2, was found in 12 archaeological turkey bones and three turkey coprolites. The third group (H3) contained all of the modern commercial turkey (both from our analyses and from GenBank data) as well as the museum specimens of the wild South Mexican turkey. These three haplogroups were separated from one another by at least 2 to 3 bp (Fig. 2).

Origins of North American Domestic Turkey Populations. The D-loop analyses indicate a clear division between the Southwest and Mesoamerican populations, with all Southwest archaeological

turkeys falling into two haplogroups (H1 and H2) and all wild *M. g. gallopavo* and contemporary domestic turkey samples falling into a third haplogroup (H3). When compared to modern wild turkey populations, these three haplogroups maintain their distinction, grouping with separate wild turkey clades (Fig. 3).

The presence of a distinct “Mesoamerican” clade (H3), including both commercially raised birds and historic wild *M. g. gallopavo*, is consistent with our historical understanding of south-central Mexico as the domestication center for the Mesoamerican turkey, with the South Mexican wild turkey as the progenitor subspecies. This result is also consistent with our historical understanding that today’s commercial birds originate from southern Mexico (4). The overall genetic uniformity of the Mesoamerican clade may be due to the limited mtDNA data available for wild *M. g. gallopavo* and the current lack of mtDNA data for the many modern domestic turkey breeds worldwide. To gain a more accurate view of the timing, specific geographic origin, and history of modern domestic turkey breeds, a more thorough genetic study must be conducted incorporating securely dated Mesoamerican archaeological samples and a wide range of modern turkey varieties (including the indigenous breeds of Mexico). Nevertheless, the current distinction between the H3 Mesoamerican clade and the H1 and H2 Southwest clades, does suggest that the turkeys exploited in the Southwest have a separate history and origin from Mesoamerican and modern domestic turkeys.

Two distinct haplogroups are found in the Southwest archaeological turkeys (H1 and H2), indicating the exploitation of two different turkey populations in the precontact Southwest. Haplogroup H1 contains the majority of the Southwest samples,

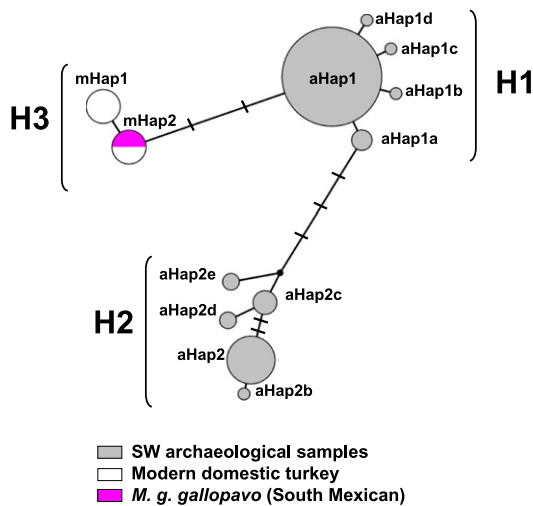


Fig. 2. Median-joining network depicting the relationship of ancient and modern D-loop haplotypes obtained in this study. Each node depicts a separate D-loop haplotype, and node sizes are proportional to haplotype frequencies in the data set. Lines between nodes represent a single nucleotide change, except where perpendicular hashes represent single changes. Gray areas within H1 and H2 nodes indicate haplotypes recovered from Southwest archaeological bone and coprolite samples. In H3, the pink area indicates haplotypes recovered from historic *M. g. gallopavo* skin samples, whereas the white areas indicate haplotypes obtained from modern commercially raised turkey samples extracted in this study.

and its haplotypes are closely related (although not identical) to those occurring in present-day Eastern (*M. g. silvestris*), Rio Grande (*M. g. intermedia*), and Florida (*M. g. osceola*) wild turkey populations that currently occur outside the Southwest (Fig. 4 and Fig. S1) (15, 16). The most common H1 haplotype observed in the archaeological samples (aHap1) also occurs at a low frequency (<4% of tested individuals) in present-day populations of Merriam's wild turkeys (Fig. 3), indicating some limited introgression with Merriam's populations in the past (see *SI Text* for detailed discussion). Haplogroup H2, on the other hand, contains sequences identical, or closely related, to haplotypes typical of contemporary populations of Merriam's wild turkey (*M. g. merriami*) and Gould's wild turkey (*M. g. mexicana*), the local wild turkeys of the Southwest and northwestern Mexico, respectively. The presence of these two distinct haplogroups within the Southwest archeological turkey samples is consistent with a scenario in which prehistoric farmers were primarily exploiting a turkey population initially introduced from outside the region (H1), in addition to local wild turkeys (H2). Alternatively, the exploitation of a more heterogeneous prehistoric population of Merriam's turkeys in the Southwest, comprising both H1 and H2 haplotypes, also remains a possibility.

Of the two clades within the Southwest, haplogroup H1 arguably displays the genetic signature of a domestic breed. This group includes the majority of the Southwest archaeological samples (85.3%), and demonstrates remarkable genetic uniformity, with 116 of the 143 sequenced archaeological samples sharing a single haplotype (aHap1). The uniformity of the H1 group points to a severe genetic bottleneck and breeding isolation most certainly associated with the domestication process (20). Archaeologically, the evidence for turkey husbandry such as turkey pens, turkey droppings, eggshells, increased number of turkey remains, and/or gizzard stones are found at sites in conjunction with H1 samples (2). The H1 haplogroup is distinct from the primary Merriam's clade, suggesting that indigenous wild birds (*M. g. merriami*) were not the progenitors of these Southwest domestic flocks. Instead, the phylogeographic,

archaeological, and ancient DNA evidence support the introduction of H1 turkeys into the Southwest as early as the Basketmaker II period (200 BC–500 AD) from a progenitor population genetically similar to present-day *M. g. silvestris* and/or *intermedia*.

Wild Progenitor for Southwest Domestic Turkeys. On the basis of the mtDNA evidence collected in this study, it is not possible to assign the wild progenitor of the H1 Southwest domestic turkey to either *M. g. silvestris* or *M. g. intermedia*. Although present-day Eastern and Rio Grande wild turkeys can usually be differentiated phenotypically (21), mtDNA analysis indicates that the subspecies do not form distinct phylogenetic clades (15). The lack of strong phylogeographic patterning may be in part, a result of drastic changes in the numbers and habitats of North American wild turkeys over the last century, including decimation of wild turkey populations beginning in the 16th century and culminating with increased hunting during the 1930s depression. Moreover, the trap-and-transplant programs implemented by the National Wild Turkey Federation 1950s conservation movement transplanted birds from some subspecies far outside their natural range. However, even "relic" populations of wild turkeys, with no history of extirpation or human-mediated reintroductions, show limited phylogeographic subspecies patterning (15, 16), which may also reflect a postglacial colonization into a relatively continuous habitat, especially for the Eastern and Rio Grande subspecies (15). These two subspecies do not cluster in monophyletic clades, but share several common haplotypes with each

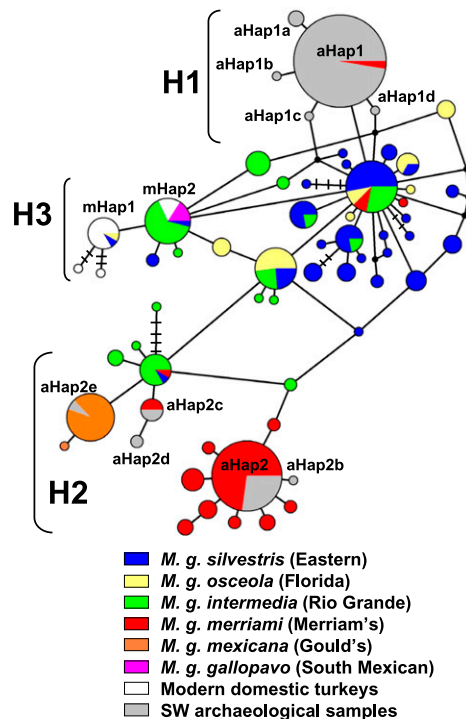


Fig. 3. Median-joining network displaying the relationships between the obtained D-loop haplotypes and available domestic and wild turkey reference sequences (15, 16). Solid colors represent haplotypes observed in modern wild turkey populations (obtained from GenBank), whereas the gray areas represent the 12 haplotypes obtained from the Southwest archaeological bones and coprolites. In H3, the pink area indicates haplotypes recovered from historic *M. g. gallopavo* skin samples, whereas the white areas indicate modern domestic turkey haplotypes extracted in this study, as well as those obtained from GenBank (identified only to the species level as *M. gallopavo*).

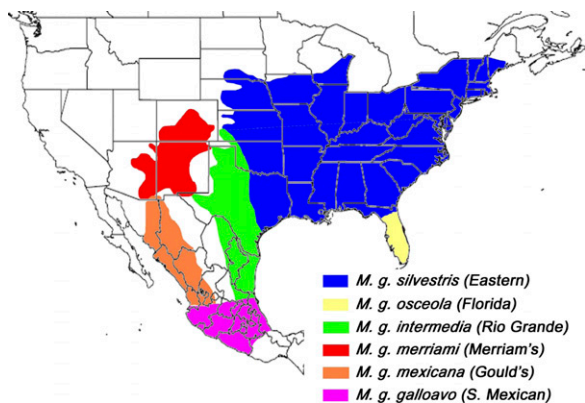


Fig. 4. Historic range of the wild turkey subspecies in North America (5).

other and with their neighboring subspecies (15) (Figs. 3 and 4 and Fig. S1).

Because the H1 wild progenitor appears related to both the Eastern and Rio Grande wild turkey, it is extremely difficult to pinpoint the origin of the H1 birds using phylogeographic analysis alone. Although it is likely that the H1 turkeys originated from an area east/southeast of the Southwest United States (within the historic range of the Eastern and Rio Grande wild turkey), these two subspecies occupy an enormous geographic range. Currently, the Rio Grande wild turkey (*M. g. intermedia*), ranges from northern Veracruz and Tamaulipas in Eastern Mexico, through to Texas, Oklahoma, and into southern Kansas (5, 22), whereas the Eastern wild turkey (*M. g. silvestris*) is found throughout eastern North America, as well as the oak-hickory forests of the Midwest (23, 24) and over much of the plains (Fig. 4).

Currently, Eastern/Rio Grande wild turkeys range only near the eastern peripheries of the Southwest states. However, there is no reliable data concerning the prehistoric distribution of these subspecies. If the ranges of Eastern or Rio Grande turkeys extended farther west in the past, populations with relatively high frequencies of H1 haplotypes may have been available for domestication somewhere within the Southwest (see *SI Text* for further discussion). There is little evidence, however, to support gene flow between Merriam's wild turkey and populations of other subspecies, as would have occurred if ranges overlapped. Merriam's wild turkey is currently geographically isolated from neighboring Eastern and Rio Grande turkeys by tracts of unsuitable habitat. Moreover, the genetic distinction between Merriam's wild turkey and the geographically closest subspecies suggests that Merriam's has been genetically isolated for a long period, perhaps even since the Late Pleistocene (15, 25). Therefore, the data more strongly suggest that the H1 turkeys were introduced into the Southwest through human-mediated exchange of domestic (or at least captive) birds.

Archaeological investigations have not yet indicated a possible origin for these H1 turkeys. Although maize and squash agriculture seems to have moved into the Southwest from central Mexico via northwestern Mexico (ca. 2100 BC) (26, 27), the conspicuous lack of turkey remains in Early Agricultural sites of northwestern Mexico, and southern Arizona and New Mexico indicates that turkeys did not enter the Southwest via this same route (2). Instead, the earliest evidence for turkeys seems to appear within archaeological sites distributed around the north-central Southwest, often in conjunction with compelling evidence for turkey husbandry (e.g., on-site turkey dung, eggshells) (28). Archaeological explorations have not yet uncovered evidence for turkey capture or domestication on the eastern/southeastern peripheries of the Southwest, although this may change with future investigations.

Although the current data may not be able to pinpoint the geographic origin of the Southwest H1 domestic lineage, the DNA data generally rule out two previously proposed wild progenitors, i.e., *M. g. gallopavo* from south-central Mexico, and *M. g. merriami*, from the Southwest. Thus, the results of this study significantly redirect archaeological and genetic inquiry into locating the source of the Southwest domestic turkey. Identifying the origin of the H1 birds may initially require a more thorough analysis of genetic variability within modern populations of Eastern, Rio Grande, and Merriam's wild turkey, in addition to further ancient DNA analysis of archaeological and historic turkey samples, both from the Southwest and neighboring regions. Although our analysis indicates the H1 haplotypes are closely related to those found in present-day Eastern and Rio Grande wild turkey populations, specific H1 haplotypes have not yet been found in these modern populations. This may well be a result of limited sampling within northern Texas, northeastern Mexico, and western Oklahoma and Kansas. Identifying the H1 haplotypes within modern populations would help guide additional archaeological investigations while ancient DNA testing on additional historic and archaeological turkey remains would refine the precontact phylogeographic pattern. A few alleged *M. gallopavo* remains have been identified in Archaic (preagricultural) sites within the Southwest and neighboring regions (29); ancient DNA analysis of these isolated finds may be key in tracking the introduction of the H1 turkeys into the Southwest.

Number of North American Turkey Domestication Centers. Although the origin of the Southwest domestic turkeys remains unconfirmed, the clear genetic distinction between the Southwest (H1) and Mesoamerican (H3) turkeys supports two incidences of turkey domestication involving two geographically distinct progenitor populations, one involving ancestral *M. g. silvestris* and/or *intermedia* (origin as yet unknown), and the other with *M. g. gallopavo* in south-central Mexico. The Southwest H1 and Mesoamerican H3 turkeys are distinguished by two to three D-loop polymorphic sites, and the current data display no shared haplotypes between the two varieties. Two biologically independent occurrences of turkey domestication do not seem unlikely and may have been facilitated by cultural knowledge relating to bird capture and breeding exchanged throughout these areas of North America. This is convergent with practices in the Old World, where multiple domestication centers have been recorded for several domesticates e.g., cattle, donkeys, pigs, and sheep (30, 31). However, the support for two turkey domestication centers is weakened somewhat by the relative paucity of comparative data from *M. g. gallopavo* and this subspecies' relative geographic proximity to *M. g. intermedia* in northeastern Mexico. A more thorough understanding of the range of mtDNA variation present in both precontact wild and domestic *M. g. gallopavo* populations will be needed to evaluate whether there may have been a single common geographic origin for both the Southwest and Mesoamerican "breeds" followed by founder effects and genetic drift as turkeys were spread throughout the two regions.

Southwest Turkey Breeding. The genetic analysis of the ancient bones and coprolites also reveals information concerning the sophistication of Southwest turkey breeding and the trade of domestic stocks. The genetic uniformity of the turkeys within the Greater Southwestern culture area for well over a millennium is remarkable and represents robust evidence for the intensive breeding of a single population through time. The DNA evidence from this study indicates that the same matriline of domestic turkey predominates from the earliest periods of domestication (ca. 200 BC–AD 450), through the peak of turkey husbandry (AD 1200–1500), and likely until the decline of domestic turkey stocks in the 18th and 19th centuries. At its

maximum extent, this domestic lineage occurred over several thousand square miles, from Tonto National Monument in Southern Arizona, to Pecos Pueblo in east-central New Mexico, and north to the San Juan region of southwest Colorado and southeast Utah, incorporating the territories of several cultural traditions including the Ancestral Puebloan (Anasazi), Salado, Mimbres, and Mogollon. The uniformity of the evidence indicates trade of turkeys between different linguistic and cultural groups and the intensive husbandry of a single turkey breed.

Exploitation of Merriam's Wild Turkey. Despite a strong focus on the exploitation of H1 domestic birds, 14.7% of the Southwest archaeological samples were identified as Merriam's wild turkey (H2), demonstrating that the local subspecies was also exploited. Previously, the lack of paleontological or archaeological wild turkey remains in the Southwest before 200 BC had led some researchers to assume that wild turkeys were not endemic (32). Because the earliest evidence that turkeys were being kept at human habitation sites was also the earliest evidence for the presence of turkeys in the region, some researchers hypothesized that modern Merriam's wild turkey populations represented the feral descendants of imported domestic turkeys (28).

The genetic data demonstrates that the domestic H1 haplogroup is distinct from the primary Merriam's clade, thus falsifying the hypothesis that extant Merriam's wild turkeys are simply descendants of escaped domestic birds. Archaeologically, small numbers of H2 (Merriam's) types appear in early contexts along with the H1 domesticates. Analysis of the more conserved cytochrome *b* gene also demonstrated a clear distinction between the H2 Southwest types and all other analyzed turkeys, providing strong support for two distinct ancestral clades—those native to the Southwest (i.e., *M. g. merriami* and possibly *mexicana*) and those originating outside the Southwest (including *M. g. silvestris*, *intermedia*, and *gallapavo*) (Fig. S2). Thus, the combined genetic and archaeological evidence supports the probable long-term presence of Merriam's turkey in the upland Southwest. Merriam's may have been present as early as 8000 BP, when suitable turkey habitat became available on the Colorado Plateau and Mogollon Rim (25).

A limited amount of introgression between domestic birds and local wild turkeys is supported by the presence of the predominant ancient domestic haplotypes (aHap1) in present-day Merriam's populations (albeit at a very low frequency) (15). This gene flow was likely the result of escaped domestic hens being incorporated into local wild populations. Introgression could have occurred at any time after the first appearance of domestic turkeys in the Southwest, but conditions in the late AD 1200s would have favored this process, as Ancestral Puebloan groups migrated out of the Colorado Plateau, perhaps abandoning some of their flocks to fend for themselves (29).

DNA analysis alone cannot determine the domestic or wild status of each archaeological individual. The occurrence of H2 bones at our sample of sites could have resulted from hunting wild birds, from confining captured birds at habitation sites, or from rearing clutches of eggs found in the locality. Expedient use of local birds is supported by the observation that of the 38 archaeological sites sampled, all but 2 of the 10 sites that yielded H2 bone and coprolite specimens are located within or near pinyon-juniper woodland and/or ponderosa pine forest, the natural habitat of Merriam's wild turkey. The wild status of the birds may be further supported by the relatively wide geographic distribution of the H2 samples and the low frequency of H2 birds within each site, as well as within the total archaeological sample (<15%). Despite the fact that the H2 birds in our samples have a persistent presence throughout all time periods, their low numbers suggests they were not major constituents of domestic flocks despite their availability through hunting or capture.

Our best evidence that “wild” birds were being kept at habitation sites comes from the H2 coprolites found at Turkey Pen Ruins in Utah, indicating that H2 birds were present and presumably confined at the site. These coprolites occurred in a thick dry midden dating almost entirely to the Basketmaker II period (ca. 200 BC–AD 450) with one H2 specimen appearing in the earliest dated stratum (Fig. S3 Table S2). Thus, the capture and provisioning of local wild birds may have been synchronous with the introduction of the domestic birds into the region. A better understanding of the nature, timing, and extent of early wild turkey exploitation will require genetic analysis of securely dated bones and/or coprolites from additional Early Agricultural sites. Additionally, investigating whether wild H2 birds were being confined and provisioned in conjunction with domestic birds must be addressed through detailed analyses of archaeological contexts, isotopic data from bones, and palynological and macrofloral evidence from coprolites.

Conclusions

Domestication is a complex process, with human–animal interactions that vary considerably in terms of their intensity and their degree of human intervention (33). The ancient DNA and archaeological evidence collected in this study reveals a wide range of past human–animal interactions within the Southwest United States, ranging from the hunting and/or capture of local wild turkeys, to the intensive husbandry and breeding of an imported domestic turkey lineage. Moreover, the DNA data indicate this Southwest domestic turkey lineage (H1) was maintained and propagated for well over a millennium, despite significant shifts in the geographic distribution and settlement patterns of Southwestern farming populations. This long history of turkey use undoubtedly reflects the economic and symbolic importance of domestic turkey for the Ancestral Puebloans, and other precontact Southwestern cultures.

This in-depth study presents conclusive evidence for the domestication of an indigenous North American animal. Moreover, as one of the few indigenous domesticates, the turkey represents an important case study through which to examine New World animal domestication in general. Previous DNA studies have exposed multiple domestications of Old World animals such as cattle, pig, sheep (30), and this study supports a similar multicenter model for the New World. The DNA data point to at least two occurrences of turkey domestication in precontact America, one involving the South Mexican wild turkey, likely in south-central Mexico, and a second involving Rio Grande/Eastern wild turkey populations, with a subsequent introduction of domesticated stocks into the Southwest proper. In addition to significantly redirecting future research into North American domestication centers, this extensive study demonstrates the complexity and sophistication of ancient husbandry and breeding practices for one of the New World's few domesticated animals.

Materials and Methods

Samples. DNA analysis was conducted on 200 turkey samples, including 149 archaeological turkey bones from 36 Southwest United States and one North Mexican archaeological sites (Table S3), and 29 turkey coprolites from the Turkey Pen Ruins site (42SA 3714), Grand Gulch, San Juan County, southeast Utah (Table S2). The sample also included 10 historic wild *M. g. gallapavo* specimens collected from Michoacan De Ocampo and Veracruz-Llave, Mexico, in 1903 and subsequently curated at the Smithsonian Institution (Table S4) and 12 commercially raised turkey samples obtained from a turkey producer on Vancouver Island, BC, Canada, and from grocery store meats in both the United States and Canada (Table S3).

DNA Extraction, Amplification, and Sequencing. Ancient DNA extraction was conducted in two dedicated ancient DNA laboratories, one located at Washington State University, Pullman, WA (WSU) and the other at Simon Fraser University in Burnaby, BC, Canada (SFU), using strict contamination

control protocols for the extraction of degraded DNA samples. At WSU, 0.09–0.34 g of coprolite material or 0.01–0.05 g of toe pad clippings were extracted according to published protocols (12), whereas at SFU, \approx 0.5 g of each archaeological bone sample was prepared and extracted according to published protocols (13, 14) (*SI Text*). The modern turkey samples were extracted using DNeasy blood and tissue kit or the DNA investigator kit (Qiagen), according to the manufacturer's instructions in separate laboratories from the ancient samples.

Overlapping primer sets were designed to obtain a 438-bp hypervariable portion of the turkey mitochondrial D-loop and 165 bp of the turkey cytochrome *b* gene (Tables S5 and S6). The obtained electropherograms were visually examined, aligned, and compiled using ChromasPro software (www.technelysium.com.au) or Sequencher 4.8. Sequences were visually edited and base pair ambiguities were examined using ChromasPro software; multiple sequences from the same bone sample were also compiled into consensus sequences using ChromasPro.

Obtained sequences from both labs were later truncated to 438 bp (position 15567–16004, on the basis of complete mtDNA genome of GenBank specimen EF153719) to make them comparable to data in Mock et al. (15). No polymorphic areas were noted in sequences surrounding these 438 bp, thus truncating the sequences did not eliminate any potentially useful phylogenetic information. Complete sequences were obtained from 159 samples (Table S2, S3 and S4) and combined with 276 *Meleagris* GenBank entries to generate a combined data set of 435 individuals.

Multiple alignments of the consensus sequences and published *Meleagris* mtDNA reference sequences were conducted using ClustalW (34) through BioEdit (35) and inspected by eye to verify the alignments. Phylogenetic analysis was conducted using MEGA 3.1 software (18) and MrBayes 3.1.2 (19). Median-joining networks were created using Network (v. 4.1.1.2) (17).

mtDNA Data Analysis. The authenticity of the analyzed data was secured by multiple criteria, including: (i) the use of dedicated ancient DNA facilities; (ii)

a vigorous decontamination protocol of the bone samples before DNA extraction; (iii) the inclusions of blank extracts and PCR negative controls; (iv) multiple haplotypes were obtained within the study as a whole, as well as within most extraction batches; (v) the two most common ancient haplotypes (aHap1 and aHap2) were obtained in two separate laboratories, using different extraction and amplification protocols and different material types (bone and coprolites); and (vi) all sequences indicate the bones, coprolites, and skins are *M. gallopavo*. Repeat extractions were conducted for 21 bones, and repeat amplifications were conducted for an additional 27 samples, including all samples with unique or unusual haplotypes. Repeat extractions were conducted on three coprolite samples and on eight *M. g. gallopavo* specimens. Consistent results were obtained for all replications.

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