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Reitz, Elizabeth, Speller, Camilla Filomena orcid.org/0000-0001-7128-9903, McGrath, Krista et al. (1 more author) (2016) A sixteenth-century turkey (Meleagris gallopavo) from Puerto Real, Hispaniola. Journal of Archaeological Science Reports. pp. 640-646. ISSN 2352-409X

https://doi.org/10.1016/j.jasrep.2016.05.050

3/7/16

Full Title: A sixteenth-century turkey (*Meleagris gallopavo*) from Puerto Real, Hispaniola

Short Title: A sixteenth-century turkey (*Meleagris gallopavo*) from Hispaniola

Elizabeth J. Reitz[a], Camilla Speller[b], Krista McGrath[b], and Michelle Alexander[b]

[a] Elizabeth J. Reitz, Georgia Museum of Natural History, University of Georgia, Athens, Georgia, USA, E-mail: ereitz@uga.edu

[b] Camilla Speller, BioArCh, Department of Archaeology, University of York, York, UK YO10 5DD, E-mail: camilla.speller@york.ac.uk

[b] Krista McGrath, BioArCh, Department of Archaeology, University of York, York, UK YO10 5DD, E-mail: krista.mcgrath@york.ac.uk

[b] Michelle Alexander, BioArCh, Department of Archaeology, University of York, York, UK YO10 5DD, E-mail: michelle.alexander@york.ac.uk

Corresponding Author: Elizabeth J. Reitz, Georgia Museum of Natural History, 101 Cedar Street, University of Georgia, Athens, Georgia, 30602-1882, USA. E-mail: ereitz@uga.edu. 1-706-542-1464.

Abstract

A single turkey (*Meleagris* spp.) coracoid was identified from Puerto Real, a Spanish colonial town founded in 1503 on the north coast of Hispaniola and destroyed in 1579. Turkeys are not indigenous to Hispaniola, but wild turkeys were widespread in lands bordering the northern Gulf of Mexico and domestic turkeys were common in parts of Mexico. A wild turkey (*M. gallopavo silvestris*) at Puerto Real might be indirect evidence that wild turkeys were sent to Europe in the early to mid-1500s from the northern coast of the Gulf of Mexico. If the Puerto Real individual is a domestic South Mexican turkey (*M. g. gallopavo*), however, this would confirm that domestic turkeys were present in the Caribbean archipelago shortly after 1492. Ancient mitochondrial DNA D-loop analysis confirmed the identification of *M. gallopavo*, with a haplotype most consistent with a Mesoamerican origin. Isotopic evidence suggested a reliance on C_4 plants, likely maize (*Zea mays*), rather than a typical wild turkey diet high in C_3 plants. Together, the biomolecular evidence suggests this turkey traces its lineage to Mesoamerica, and is part of the larger post-Columbian merger of diverse cultural traditions.

Keywords

Meleagris gallopavo; Puerto Real; ancient DNA analysis; stable isotopes; Columbian Exchange

1. Introduction

In 1979, a single turkey (*Meleagris* spp.) coracoid was recovered from the Spanish colonial town of Puerto Real, a short-lived Spanish town founded on the north coast of Hispaniola in 1503 and destroyed in 1579 (Fig. 1). Turkeys are not indigenous to Hispaniola, but wild turkeys were widespread in lands bordering the northern Gulf of Mexico and domestic turkeys were ubiquitous in many parts of Mexico (Fig. 2). Although represented by a single specimen, this turkey provides a new perspective into the global exchange of products among the Americas, Europe, Asia, and Africa associated with post-Columbian voyages sponsored by European states, an exchange known as the "Columbian Exchange" (Crosby, 1972, 1986). An Eastern wild turkey (*M. gallopavo silvestris*) at Puerto Real would be indirect evidence that wild turkeys were sent to Europe in the early to mid-1500s from this Gulf coast region; a domestic South Mexican turkey (*M. g. gallopavo*) would confirm that domestic turkeys were present in the Caribbean archipelago shortly after 1492, potentially *en route* to Europe. This paper reviews the osteological, historical, and biomolecular evidence for the origin of this turkey to place it within the larger trade network of the early sixteenth century. In many ways, this specimen symbolizes the rapid merger of diverse cultural influences initiated in 1492 that characterized the late fifteenth and early sixteenth centuries.

1.1. Puerto Real

Puerto Real was an early sixteenth-century town in what is now Haiti (Deagan, 1995; Lyon, 1995). It was one of the first 15 towns founded on the island of Hispaniola in 1503. Nearby Puerto Plata was officially the last port of call for ships sailing from the Americas to Spain until 1515, when Havana, Cuba, was founded (Sauer, 1969:154). Puerto Real itself was a busy port from which slaves and cattle products such as hides and tallow were sent to Europe. Much of this trade was with Portuguese, Dutch, French, and English ships that called at Spanish ports on northern Hispaniola. This was a violation of Spanish mercantile policies that gave the Spanish Casa de Contratación a monopoly on the American trade. After 1515, all Spanish ships were to rendezvous in Havana to join the annual convoy returning to Spain. Repeated efforts to counteract pirates and corsairs, limit the extensive illegal trade with foreign vessels, and exert more control over commerce on Hispaniola failed. The Crown ordered Puerto Real and the other northern ports abandoned in 1578 and Puerto Real was destroyed by Spanish officials in 1579. Puerto Real is a cautionary tale that orders to trade only with Spanish ships and to join the Havana fleet each fall were not followed, at least not by non-Spanish vessels.

Excavations at Puerto Real between 1979 and 1985 produced several zooarchaeological surprises (Deagan, 1995). Two of these were the dominance of very large cattle, as well as wild animals such as Allen's hutia (Capromyidae: *Isolobodon portoricensis*) and turtles (Deagan and Reitz, 1995; McEwan, 1983, 1995; Reitz and McEwan, 1995; Reitz and Ruff, 1994). This combination of local and Eurasian animals characterized much of the early colonial effort in the Americas. Two specific specimens are noteworthy. One of these is a cheek tooth from a round-tailed muskrat or Florida water rat (Muridae: *Neofiber alleni*) and the other is the turkey coracoid (*Meleagris* spp.; Ewen, 1987:195; McEwan, 1983; Reitz and McEwan, 1995). The Puerto Real turkey is not the only one reported from Hispaniola, Stephen Cumbaa identified a turkey from the Convento de San Francisco, a sixteenth-century monastery in Santo Domingo on the southern coast of Hispaniola in what is now the Dominican Republic (Cumbaa, 1975:63). The question is: did the muskrat and turkey ultimately derive from the same region of the American mainland or did these two individuals, or their ancestors, originate in different regions?

Neither muskrats nor turkeys are indigenous to the Caribbean. Both animals likely were part of a trade in which exotic plants and animals were sent to Europe from the Americas, though not necessarily through Puerto Real. Prior to 1515, when ships were order to sail from Havana, however, all exotic animals likely did pass through port towns on Hispaniola on their way to Europe. The Puerto Real muskrat and turkey did not reach Europe, of course, but died at Puerto Real. It is possible that neither of these specific individuals were even destined for Europe. The turkey, in particular, may have been born and raised at Puerto Real for local use.

The muskrat plays a role in this story only because it is the reason the northern coast of the Gulf of Mexico might be the source of the Puerto Real turkey. Muskrats are restricted to the southeastern region of what is now the United States, specifically southern Georgia and peninsular Florida (Fig. 2; Hall and Kelson, 1959:754). The Puerto Real muskrat clearly testifies to direct or indirect contact between the northern Gulf coast and Hispaniola. Spanish explorers and European cartographers were familiar with Florida, the Florida Straits, and the northern Gulf coast as early as 1502 and Spain sponsored numerous expeditions to the region, most of which sailed to Florida from the Greater Antilles, initially only from Hispaniola, and later from Puerto Rico and Cuba. The 1512 expedition was led by the Governor of Puerto Rico, Juan Ponce de León. Eventually, the Governor of Cuba, Pedro Menéndez de Avilés, successfully established the first European colonial town north of Mexico, naming it San Agustín, or St. Augustine. Menéndez reported his success in routing French colonists from Spanish Florida to the Audiencia of Santo Domingo on Hispaniola via a courier sent from St. Augustine to Puerto Real (Lyon, 1995). Thus, there were numerous opportunities for muskrats, as well as wild turkeys, to be brought from Florida to Puerto Real and either these same birds or their offspring sent on to Europe.

2. Wild and domestic turkeys

The turkey is a much more complicated story, made more so by the taxonomic classification that prevailed when this study began. In the 1970s, the nomenclature used by some ornithologists divided turkeys into two genera: *Agriocharis ocellata*, the ocellated turkey (now termed *Meleagris ocellata*), and *Meleagris gallopavo*, commonly referred to as the northern or wild turkey. The first question that needed to be answered was whether the archaeological specimen could be attributed to *Agriocharis* or *Meleagris*. Taxonomic revision removed the possibility that it was an *Agriocharis* and the specimen was published with the *Meleagris* attribution (Reitz, 1986; Reitz and McEwan, 1995).

The ocellated turkey's range is confined to the Yucatan peninsula (Schorger, 1963, 1966; Steadman, 1980:158). The wild turkey subspecies, however, are much more widely distributed. They are found in most of what is now the eastern United States, portions of the southwestern United States, as well as eastern and western portions of Mexico. They are traditionally divided into six regional subspecies: Eastern (*M. g. silvestris*), Florida (*M. g. osceola*), Rio Grande (*M. g. intermedia*), Merriam's (*M. g. merriami*), Gould's (*M. g. mexicana*), and the (most likely) extirpated South Mexican turkey (*M. g. gallopavo*) (Schorger, 1966:43, 49). It is the latter (*M. g. gallopavo*) which is thought to have been domesticated ca. 800 BCE within the Mexican part of the wild turkey's range (Schorger, 1966; Speller, 2014), while either *M. g. silvestris* and/or *M. g. intermedia* seem to be the progenitors of the Puebloan domestic turkey of the American Southwest (Speller, 2014; Speller et al., 2010). In Mesoamerica, domestic turkeys were raised for food and tribute to local leaders, spreading from south-central Mexico into the Yucatan Peninsula as early as CE 100 (Thornton et al., 2012), and becoming more widespread in other parts of Central America just before European contact (Crawford, 1992).

Along the northern Gulf coast, local populations of Florida, Eastern, and Rio Grande wild turkeys were not domesticated (Schorger, 1966:68), though wild birds were exploited. Thus, if the Puerto Real turkey, or its ancestors, were derived from northern Gulf coast populations, it was likely a tamed or captive wild bird; if it was from Central America, it was likely from a domestic population.

Turkeys were well-known to Spanish, French, and English colonists and were frequently listed among the fruits of the land. Turkeys were known in the Spanish world within a decade of Columbus's first voyage (Schorger, 1963) and were in Spain by 1519 (Schorger, 1966:8-9, 463). Columbus reported both turkeys and Muscovy ducks (Anatidae: *Cairina moschata*) when he visited the Yucatan coast in 1502 (Sauer, 1969:1300; Schorger, 1963). Licenciado Alonso Zuzao wrote to Emperor Carlos V in 1514 that Spanish explorers in Panama were served turkey. Both birds were likely the domestic Central Mexico subspecies *M. g. gallopavo*, based on what is known about the range of *ocellata* and *gallopavo* subspecies and biomolecular evidence (Sauer, 1969:249, 274-275; Thornton et al., 2012). In 1518, Zuazo wrote Carlos V that he was sending turkeys from Hispaniola (Sauer, 1969:209) and a letter cited by Schorger (1966:464) from Carlos V refers to turkeys received in Seville from Hispaniola. Carlos V was the Holy Roman Emperor. As Carlos V, he ruled over extensive domains in central, western, and southern Europe. As Carlos I, he ruled the Spanish Empire. Once turkeys reached Seville, Spain, they could quickly spread throughout the Hapsburg Empire. Given the illegal trade along the northern coast of Hispaniola, turkeys likely also traveled aboard vessels of other nationalities.

3. Materials and methods

Occupational dates for the town of Puerto Real are well-known through documentary sources. The turkey specimen itself was recovered from Locus 33/35 (McEwan, 1983, 1995). Building debris, ceramics, glassware, and other artefacts all indicate that Locus 33/35 was largely a pre-1550 residence of a high-status Spanish household (McEwan, 1995). A fence line may have enclosed a back yard into which residents discarded trash to form a large midden. Puerto Real was an active cattle station, though Locus 33/35 was not as rich in cattle remains as other parts of the town (e.g., Deagan and Reitz, 1995; Reitz, 1986; Reitz and McEwan, 1995; Reitz and Ruff, 1994). The Locus 33/35 materials are described in detail elsewhere (McEwan, 1995).

The turkey specimen was recovered from Level 3. Levels 1 and 2 at Locus 33/35 consisted of a 2-4 cm plow zone and a 2-8 cm lens beneath the plow zone; faunal remains were not studied from these two levels. Twenty-two units were excavated in Level 3 and 20 units in Level 4, representing 88 m^2. Level 3 was a dense deposit that varied in thickness between five and forty cm. The level contained 28,339 vertebrate specimens and the remains of an estimated minimum of 96 individuals. Level 4 was the living surface during the Spanish occupation and contained 962 specimens representing an estimated minimum of 10 individuals. Other contexts at Locus 33/35 yielded an additional 809 specimens representing an estimated minimum of 21 individuals. These included the remains of a domestic cat (*Felis catus*), two horses, mules, or donkeys (*Equus* spp.), twenty-four pigs (*Sus scrofa*), ten cows (*Bos taurus*), two sheep or goats (Caprinae), and six chickens (*Gallus gallus*). All of these are post-Columbian introductions from Eurasia. More suggestive of the role of cattle in the town's export economy is the recovery of 17,032 vertebrate specimens attributed only to Unidentified Mammal but likely to be from cattle given the size of the specimens (Reitz and McEwan, 1995).

AMS dating was unavailable during the original study, and was not considered prior to the stable isotope and aDNA study because of the small amount of material available for the genetic study (3.5 g) and the documentation available for the specimen's context. All archaeological deposits are subject to admixture, but the stratigraphy of the locus and the material assemblage all suggest the specimen was recovered from a closed context.

Biomolecular and stable isotope analyses were combined with traditional osteometric analysis to determine the identity and origins of the Puerto Real turkey. The first question to be resolved was whether it was *M. ocellata* or a subspecies of *M. gallopavo*. If the specimen proved to be a subspecies of *M. gallopavo,* mtDNA sequences might further indicate whether the turkey was from the Mesoamerican domestic population or a northern Gulf coast wild population. Isotope analysis would provide information about the animal's husbandry history through its bulk diet. The diet of wild turkeys throughout their range is dominated by plants following the C_3 pathway (Stearns, 2010) whereas domesticated turkey (at least those from the southwestern United States) were provided a diet that included copious amounts of maize (*Zea mays*), a C_4 plant (McCaffery et al., 2014; Rawlings and Driver, 2010) with a distinct $\delta^{13}C$ value.

3.1. Osteometric analysis

The coracoid was compared to reference materials at the Florida Museum of Natural History (Fig. 3). Due to the pattern of breakage, the specimen could not be measured following von den Driesch's (1976) criteria. Dimension "D" (Steadman 1980:159), the distance from the head through the scapular facet, could be measured, however.

3.2. Ancient DNA analysis

The DNA analysis was conducted at the dedicated Ancient DNA Laboratory at the University of York following strict contamination control protocols. A section of the coracoid was removed using a sterilized saw blade. The sub-sample was decontaminated in 6% sodium hypochlorite for seven minutes, rinsed twice with HPLC grade water, exposed to UV light for 30 minutes on two sides, and ground into powder. Multiple DNA extractions were performed on the coracoid.

Approximately 50 - 70 mg of bone powder was combined with 2 ml lysis buffer (0.5M EDTA, pH 8.0, 0.5 mg/ml proteinase K) and incubated overnight at either 37°C or 50°C. Two silica-based spin column extraction protocols (using Qiagen minelute columns) were used to isolate the DNA. Three extracts of bone powder were prepared following the method described in Dabney et al. (2013), while a fourth was prepared using the method proposed in Yang et al. (1998), following modifications as described in Speller et al. (2010). Following extraction of the first three replicates, any remaining bone powder from the three extractions were combined, an additional 2 ml of lysis was added, incubated at 50°C, and extracted using the method described in Speller et al. (2010). Elutions from all five extractions were PCR-amplified using primers targeting the 600 base pair (bp) region of mitochondrial D-loop analyzed in previous studies of modern (Mock et al., 2002) and ancient North American turkeys (Speller et al., 2010), using overlapping primer sets, PCR reactions and annealing conditions described in Speller et al. (2010), as well some additional primers developed for this study (SI Table 1). Successfully amplified PCR products were sequenced using forward and/or reverse primers at Eurofins Genomics; obtained sequences were edited and compiled into consensus sequences using Chromas Pro (http://technelysium.com.au/), and multiple alignments were conducted through BioEdit (Hall, 1999). The Puerto Real haplotype was compared with previously published ancient and modern wild and domestic turkey haplotypes obtained from GenBank (Mock et al., 2002; Monteagudo et al., 2013; Speller et al., 2010), and median-joining networks were constructed using Network v.4.6 (Bandelt et al., 1999).

3.3. Stable isotope analysis

Collagen was extracted from the Puerto Real specimen at BioArCh, Department of Archaeology, University of York, following a modified Longin (1971) method (Brown et al., 1988). The outer

1 - 2 mm of the surface of the bone was removed with a scalpel and 320 mg demineralised in 0.6M HCl at 4ºC. The resultant collagen pseudomorph was gelatinised in pH3 solution at 80ºC for 48 hours before filtering with 5 - 8 µm Ezee® filters. The resultant supernatant was filtered through 30kDa ultrafilters (Amicon®, Millipore) to separate the higher molecular weight fraction, which was subsequently lyophilised. The collagen sample (~1 mg) was analysed in triplicate using an EA/IRMS in a GSL analyser coupled to a 20 - 22 mass spectrometer (Sercon, Crewe, UK) at the University of York. Instrument error was < 0.2‰ for both $\delta^{13}C$ and $\delta^{13}N$ calculated from repeated measurement of an internal laboratory standard (fish gelatin).

4. Results and discussion

4.1. Osteometric analysis

The distance from the head through the scapular facet was 29.4 mm. This dimension in female *M. gallopavo* is 28.0 - 34.2 mm with female *M. ocellata* being considerably smaller (Steadman, 1980:158). Males of both taxa are larger than females, but male *ocellata* are smaller than male *gallopavo*. According to this measurement, the Puerto Real specimen could be either a small female *gallopavo* or small male *ocellata*. Thus, measurements do not clarify whether the Puerto Real turkey was a domestic *gallopavo* or a wild *ocellata*.

4.2. Ancient mtDNA results

Targeting short overlapping fragments of mtDNA, 454bp of D-loop were recovered and reliably replicated, corresponding to positions 15,507 - 15,960 of the turkey mtDNA genome (Genbank Accession EF153719). Fragments greater than 150bp in length (including primer sequences) could not be regularly amplified from the extracts. This pattern of bimolecular preservation is expected for tropical climates (Hofreiter et al., 2015) and consistent with the relatively poor DNA preservation observed in other ancient DNA studies in the Caribbean (Brace et al., 2015;

Lalueza-Fox et al*.,* 2003, Mendisco et al*.,* 2015, Speller et al*.,* 2013). DNA damage also was observed through several C-> U-type damage derived miscoding lesions (Brotherton et al., 2007; Gilbert et al., 2003), with multiple sequence replicates used to confirm the consensus sequence. The fragment of mtDNA confirms the identification of the coracoid as *M. gallopavo*, differing by more than 20bp from published *ocellata* DNA sequences (Mock et al*.,* 2002).

The goal of the study was to determine whether the Puerto Real turkey was phylogenetically more similar to turkeys from Mesoamerica (*M. g. gallopavo*), or wild turkeys from the northern Gulf coast (i.e., Eastern (*M. g. silvestris*) and Florida (*M. g. osceola*) wild turkey haplotypes). We compared the Puerto Real D-loop to mitochondrial haplotypes identified in modern wild turkey populations found throughout North America (Mock et al*.,* 2002); in the absence of published ancient DNA data on pre-Columbian Mexican domestic turkeys, we compared the Puerto Real sequence to previously published sequences from Mexican wild turkeys (*M. g. gallopavo*) and modern domestic breeds descended from Mesoamerican birds (Montealgudo et al*.,* 2013; Speller et al*.,* 2010).

Previous analyses of North American turkeys identified at least three haplogroups: H1 and H2, observed in ancient and modern turkeys of the American Southwest, and H3, which included modern domestic turkey breeds, as well as Mesoamerican wild populations. The Puerto Real turkey was assigned to haplogroup H3, and the 454bp fragment was identical to haplotype mHap2 (GenBank Accession GQ303165), previously observed in modern North American and European domestic turkeys, as well as historic samples of South Mexican wild turkey (*M. g. gallopavo*) (Monteagudo et al*.,* 2013; Speller et al*.,* 2010) (SI Fig. 1). Mock's 2002 genetic survey of modern North American wild turkeys identified haplotype mHap2 in approximately 25% of modern Rio Grande turkeys (*M. g. intermedia*), which currently reside along streambordering woods and scrub of the southern Great Plains of Texas and the Gulf coast of Tamaulipas. In contrast, the mHap2 haplotype was identified only in a single Eastern wild turkey (*M. g. silvestris*), and was absent in the Florida wild turkey populations (*M. g. osceola*). Although there are no published DNA sequences from Mexican archaeological turkey bones, the dominance of mHap2 in modern domestic breeds from both Europe and North America (both originating from Mexico), as well as in Mexican wild turkeys suggests that the ancestral source population of the Puerto Real specimen lies in Mesoamerica, rather than the Florida Gulf coast. Thus, based on the DNA evidence, the turkey and the muskrat at Puerto Real appear to be ultimately derived from distinct geographic areas.

4.3. Stable isotope analysis

Table 1 presents the results of isotopic results for the Puerto Real turkey and the data is plotted in comparison to other ancient and historic period turkeys in Fig. 4. The sample passed quality indicators, with a 5.1% collagen yield a C:N ratio within 3.1 - 3.5 and acceptable %C and %N values (Ambrose, 1990; DeNiro, 1985; van Klinken, 1999).

North American wild turkeys are generally opportunistic omnivores, with a highly varied and seasonal diet (Hurst, 1992). Analysis of turkey crops and feces suggest that turkeys consume primarily hard mast (e.g., acorns, pecans, pine), soft mast (e.g., dogwood, blackberry, mulberry, blueberry), grasses, seeds, sedges, as well as insects (e.g., grasshoppers, beetles, spiders), snails, and small lizards (Dalke et al., 1942; Glover and Bailey, 1949; Hurst, 1992). These plants generally follow the C_3 photosynthetic pathway, with $\delta^{13}C$ values often less than -26‰. Although wild turkeys have been known to consume maize (e.g., when provided in self-feeders), it usually represents a winter “emergency” food used when mast or other “native” foods are scarce or difficult to access (Glover and Bailey, 1949; Schemnitz, 1956). The dominance of C_3

plants in the modern wild turkey diet has recently been demonstrated through stable isotope analysis of wild turkey feces, which have an average $\delta^{13}C$ of -26.7‰ and an average $\delta^{15}N$ value of 1.21‰ in adult birds of both sexes (Stearns, 2010).

These wild values stand in contrast to the diet of pre-Columbian domestic turkeys, which seem to have consumed a diet richer in maize. Studies by Rawlings and Driver (2010) and McCaffery et al. (2014) both demonstrate that turkeys raised by the Ancient Puebloans of the American Southwest were significantly enriched in ^{13}C compared to wild birds. Although no comprehensive isotopic studies have been published for pre-Columbian Mesoamerican domestic turkeys (see limited data in Wright [2006] for the Mayan region), our expectations were that maize provisioning also occurred throughout Mesoamerica. Thus, for the Puerto Real specimen, our expectations were that $\delta^{13}C$ values of -19 to -22‰ would indicate a “wild turkey” diet consisting primarily of C_3 plants and values closer to -10 to -6‰ would suggest provisioning with C_4 maize.

As can be seen in Fig. 4, the $\delta^{13}C$ value of the Puerto Real turkey (-12.5‰) suggest a diet rich in C_4 plants compared to wild turkeys, more similar to Puebloan domestic turkeys from the American Southwest (Rawlings and Driver, 2010) than to most ancient wild turkeys from the northern Gulf coast (Hard and Katzenberg, 2011) and other parts of North America (Guiry et al., 2012; Katzenberg, 1989; Martin, 1999) which is altogether suggestive of a domestic individual. Interestingly, the Puerto Real turkey has a $\delta^{13}C$ value similar to two turkeys recovered from the colonial Spanish Nuestra Señora del Refugio Mission in coastal Texas (Hard and Katzenberg, 2011). Although the two Texas turkeys have enriched ^{13}C compared to other “wild” turkeys tested from the northern Gulf coast, it is not known whether they represent captive birds, perhaps raised alongside chickens (*Gallus gallus*) at the site (Tennis et al., 2002), or wild turkeys with a heavy reliance on coastal savanna grasses, many of which are C_4 plants.

The $\delta^{15}N$ value for the Puerto Real specimen (12.1‰) is remarkably elevated compared to the other turkeys considered here (although one Puebloan turkey individual is close at 10.8‰). Enrichment in ^{15}N can occur due to a number of environmental factors, including temperature, aridity, coastal proximity (Amundson et al., 2003) and human land use, particularly manuring (Fraser et al., 2011). The nitrogen signature, however, is similar to those reported from chickens from fifteenth- and sixteenth-century Spain (Alexander et al., 2015), domestic fowl from Medieval England (Müldner and Richards, 2007) and terrestrial birds (chickens) from Rapa Nui (Commendador et al., 2013) the latter demonstrating particular enrichment with $\delta^{15}N$ values up to ~15‰ (Fig. 4).

Domestic fowl kept within human settlements are opportunistic feeders that supplement any direct feeding from humans with scavenging food waste and consumption of invertebrates, which tend to be enriched in ^{15}N (Markow et al., 2000). This, coupled with the likelihood of the birds being kept in an area that they will have rooted in soil enriched with their own manure, will result in elevated $\delta^{15}N$ values (Rogers, 2009). As turkeys are closely related to chickens, they may have been kept under similar management conditions at this site. Indeed, turkey and chicken remains found at the same fishing station in seventeenth- through nineteenth-century Newfoundland (Canada) were found to have similar isotopic values, suggesting similar feeding and therefore husbandry practices for these domestic fowl (Guiry et al., 2012). Puerto Real was a cattle station engaged in international trade in cattle products (Deagan and Reitz, 1985), thus additional enrichment in ^{15}N due to the influence of cattle manure and intensive husbandry is a distinct possibility.

A potential marine influence behind the $\delta^{13}C$ and $\delta^{15}N$ values for the Puerto Real turkey also cannot be ruled out, although it is notable that published data from turkeys from coastal areas do not exhibit similarly elevated $\delta^{15}N$ values (Hard and Katzenberg, 2011) and chickens on a similar island location (Commendador et al., 2013) do not demonstrate ^{13}C enrichment indicative of marine input (Fig. 4). Further analysis of $\delta^{13}C$ in lipids (Colonese et al., 2015) or compound specific isotope analysis of collagen (Corr et al., 2005) may resolve the C_4/marine issue. Ultimately, without isotopic data from other species from the same site for comparison, it is not possible to assess the relative impact of husbandry and environmental factors behind the elevated $\delta^{15}N$ value in the Puerto Real turkey.

5. Conclusion

The combined biomolecular evidence obtained from the Puerto Real specimen is most consistent with a captive-reared or domestic turkey originating from Mesoamerican stock. In terms of the original question "are the muskrat and turkey from the same region of the American mainland or did these two individuals, or their ancestors, originate in different regions?" it appears either these individuals are from different regions or can trace their ancestry to different regions. This bird, in combination with the muskrat from the northern coast of the Gulf of Mexico and aspects of the Puerto Real faunal assemblage discussed elsewhere (Deagan and Reitz, 1995; Reitz and McEwan, 1995) documents the speed with which colonists explored novel resources throughout the Caribbean and Gulf of Mexico basins. Although patterns of animal use emerging in each colony were molded in part by specific local cultural and environmental conditions, it also was shaped by interactions between colonists and Native Americans as both explored the new landscape for economic opportunities. These interactions played significant roles in shaping subsequent history.

A fundamental question pertaining to this process is whether the outcome is a mixture of several cultural strains, with roots that can be traced in a more or less linear fashion back to an original ancestry, or “a new cultural form with multiple origins and multiple active agents” (Deagan, 1998:23, 25). Archaeological evidence from early European-sponsored settlements throughout the Americas testify to the rapidity with which Native American traditions in animal use, material culture, architecture, and other aspects of daily life merged with European and African ones to form new cultural traditions that cannot be traced back to a single cultural heritage merging traditions from multiple origins and involving multiple agents (Reitz and Waselkov, 2015).

The Puerto Real muskrat and turkey are part of the much larger, more important history of colonization in the Americas. In colonial settings, where multi-group interactions and exchange occurred, this new tradition was the outcome of dynamic exchanges, reformations, and inventions. As the Puerto Real turkey clearly demonstrates, these cultural transformations occurred rapidly and had far-reaching consequences.

Acknowledgments

We wish to thank Kathleen A. Deagan and the late Charles H. Fairbanks for the opportunity to examine the vertebrate remains from Puerto Real, as well as Bonnie G. McEwan and Charles R. Ewen for access to the collections they excavated and studied. We are particularly grateful to Kathleen Deagan and Kitty Emery for permission to undertake destructive analysis of the turkey specimen. We also appreciate the assistance of Elizabeth S. Wing and the staff of the Florida Museum of Natural History. Charles H. Wood, David W. Hall, Timothy A. Olsen, Diana Matthiesen, Lee McDowell, Lawrence D. Harris, Lewis Yarlett, Victor W. Carlisle, and Rochelle Marrinan provided invaluable information. We are particularly grateful to David Steadman for permission to reproduce his line drawing in Figure 3. Figures 1 and 2 were produced by Susan G. Duser. Excavations at Puerto Real were funded by the Florida Museum of Natural History and DNA analysis was funded by a European Commission Marie Curie Action Fellowship (ORCA FP7-PEOPLE-2011-IIF 299075). Earlier versions of this paper were presented at the 48th Annual Meeting of the Society for American Archaeology, Pittsburgh (1983),“Rethinking the Encounter: New Perspectives on Conquest and Colonization” (1988) funded by the National Endowment for the Humanities; the 6th International Conference of Archaeozoology, Washington, DC (1990), and the 12th International Conference of Archaeozoology, San Rafael, Argentina.

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Appendix A. Supplementary data

SI Fig. 1: Median-joining network displaying the relationships between the Puerto Real D-loop haplotype (purple) and domestic and wild turkey reference sequences (Mock et al., 2002; Monteagudo et al., 2013; Speller et al., 2010). Each node depicts a separate D-loop haplotype, and node sizes are proportional to haplotype frequencies in the data set. The haplotype detected in the Puerto Real turkey groups within the H3 haplogroup, predominantly observed in modern domestic turkey breeds, historic South Mexican wild turkeys, and modern Rio Grande wild turkey populations.

SI Table 1: PCR primers used to amplify turkey D-loop fragment.

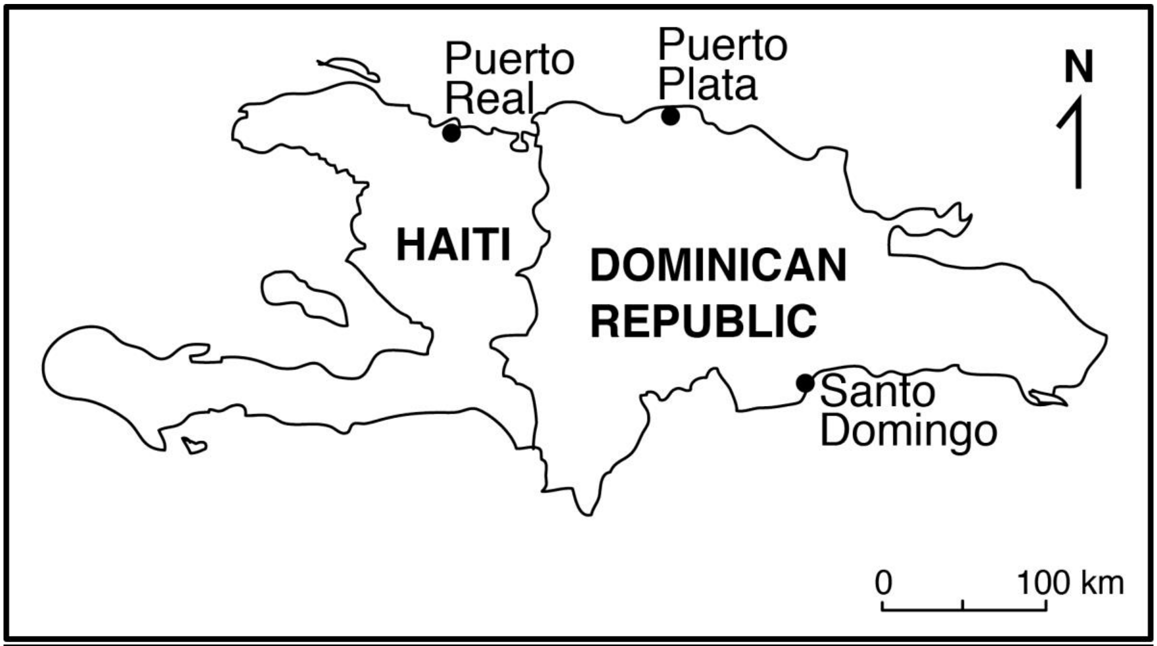


Fig. 1. Map of Hispaniola.

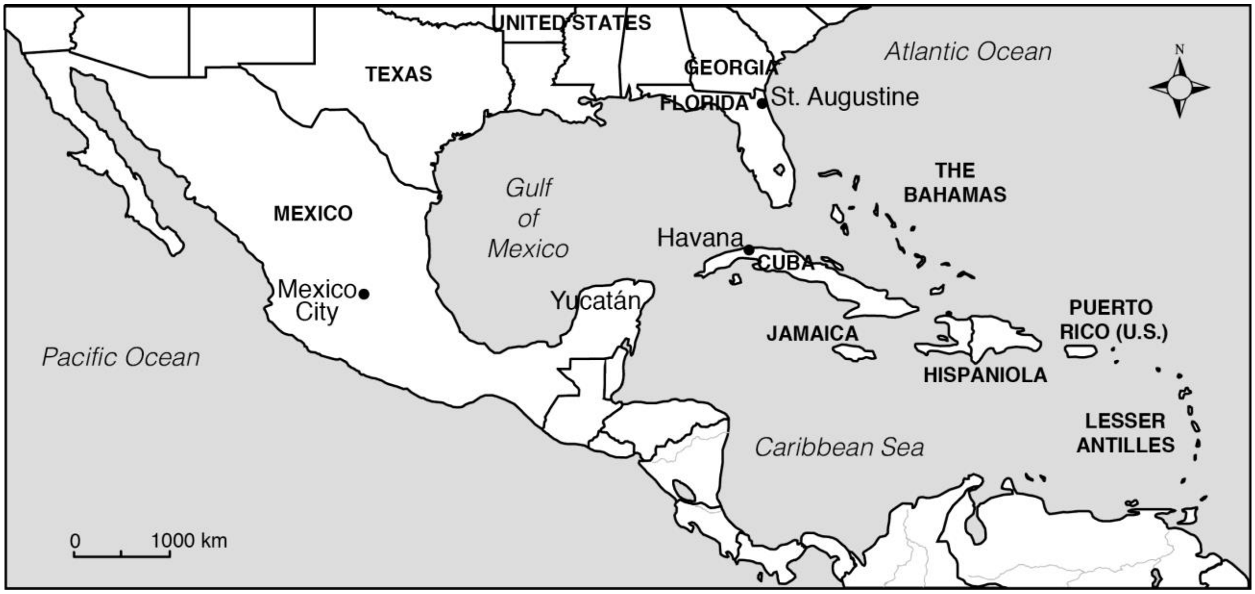


Fig. 2. Map of Caribbean Basin and Gulf of Mexico.

Fig. 3. *Meleagris gallopavo* specimen from Puerto Real, Haiti (FLMNH 03030189; FS# 2069) and dimension "D" (Steadman 1980:159). Line drawing showing dimension "D" used with the kind permission of David W. Steadman.

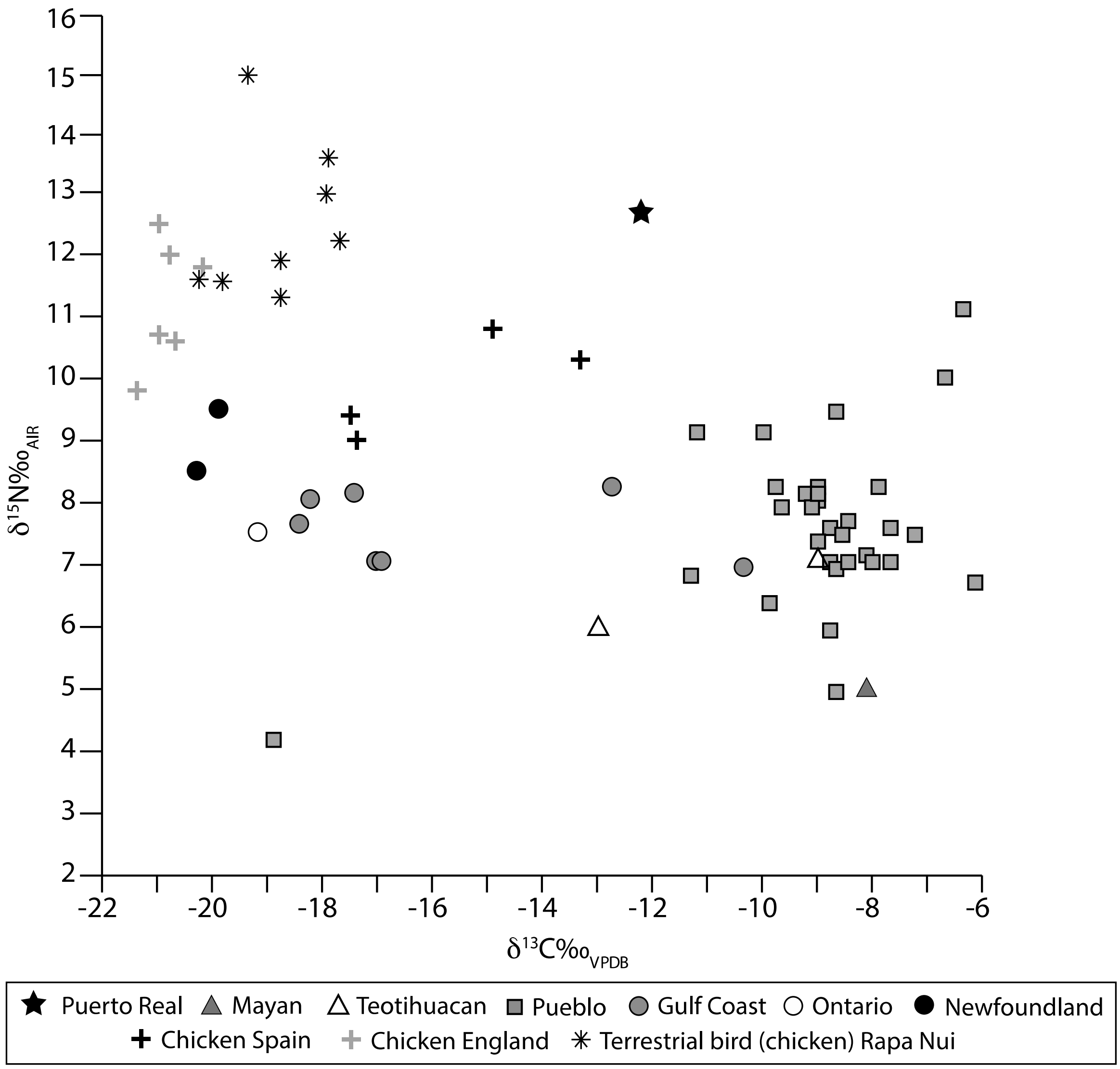


Fig. 4. Stable isotope values of the Puerto Real specimen (solid star) compared to other ancient and historic period turkeys from the Mayan region (Wright, 2006), Teotihuacan (Morales-Puente et al., 2012), Gulf Coast (Hard and Katzenberg, 2011), American Southwest (Martin, 1999; Rawlings and Driver, 2010), Southern Ontario (Katzenberg, 1989), Newfoundland (Guiry et al., 2012) and historic period chickens (crosses/stars) from Spain (Alexander et al., 2015), England (Müldner and Richards 2007) and Rapa Nui (Easter Island) (Commendador et al., 2013).

Table 1.

Stable carbon and nitrogen isotope values for the Puerto Real turkey collagen.

Sample	$\delta^{13}C$ (‰)	$\delta^{15}N$(‰)	C (%)	N (%)	C:N	Collagen yield (%)
TU4338A	-12.16	12.06	41.4	14.3	3.4	5.11

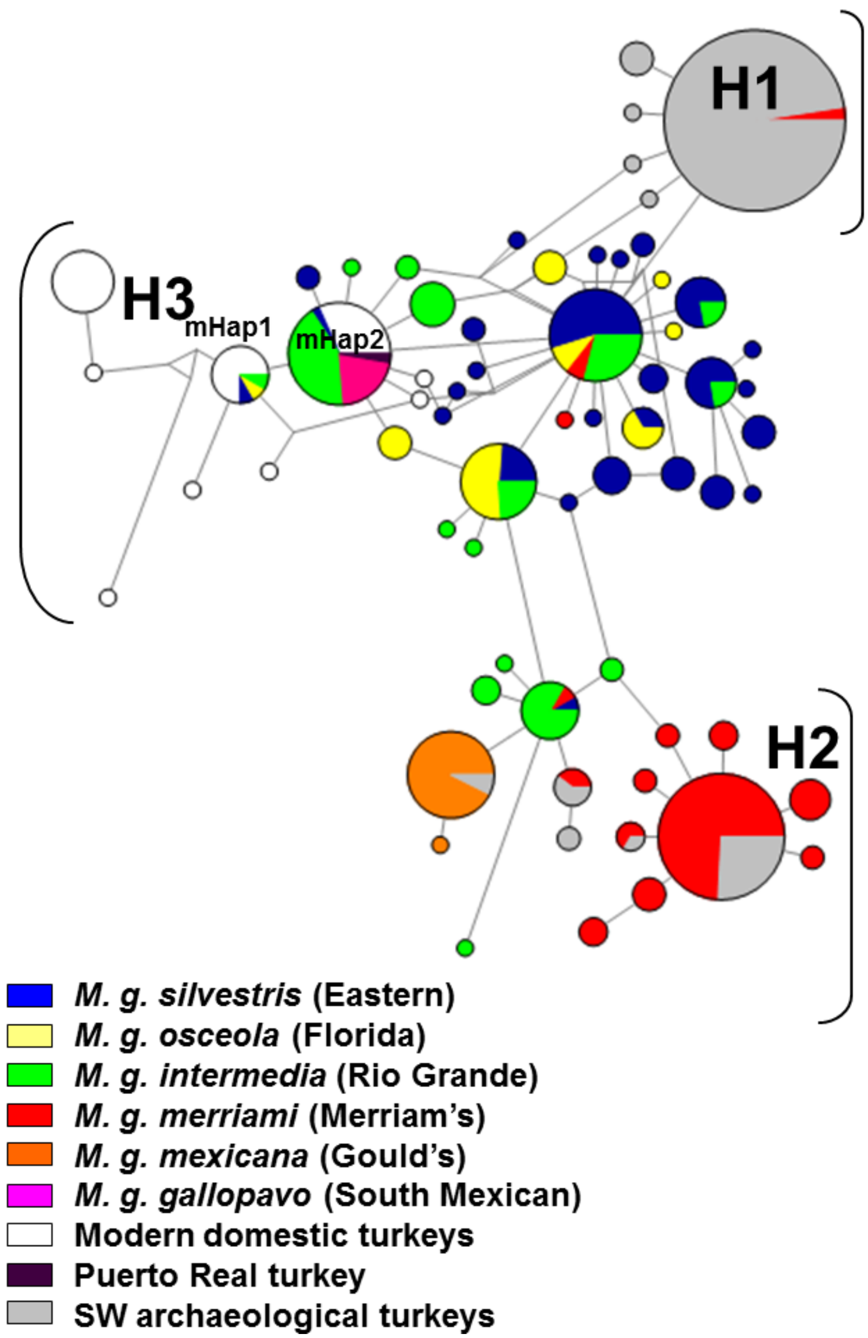
Supplementary Information

SI Table 1: PCR primers used to amplify turkey D-loop fragment.

Primer name	Coordinates*	Sequence (5′ to 3′)
TK-F2[a]	15482–15505	AATTTATTCCCGCTTGGATAAGCC
TK-F143[a]	15624–15650	GCATAATCGTGCATACATTTATATACC
TK-R156[a]	15613–15636	TGCACGATTATGCATAGTATACCC
TK-F224 [a]	15704–15729	GTAGACGGACATAACAACCTTTACCCC
TK-F315 [a]	15759–15782	ACATGCCAATGACATTAACTCCTTC
TK-R368	15818- 15845	GGTATGTCCTGTAACCATTCATGTATAT
TK- R380	15830- 15857	GTAAGATTTAGAGGTATGTCCTGTAACC
TK-R405[a]	15801–15824	TGTATATGGTCTCTTGRGGGTTGG
TK-F411[a]	15829–15854	TGGTTACAGGACATACCTCTAAATCT
TK-R420	15875-15897	CCATCTGGTACGTCGAGCATAAC
TK-R567[a]	15962–15981	GGGAAAGAATGGGCCTGAAG

F and R in the primer name denotes forward and reverse primers, respectively.

*Coordinates, numbered according to the reference sequence (GenBank accession EF153719).



SI Fig. 1. Median-joining network displaying the relationships between the Puerto Real D-loop haplotype (purple) and domestic and wild turkey reference sequences (Mock et al., 2002; Monteagudo et al., 2013; Speller et al., 2010). Each node depicts a separate D-loop haplotype, and node sizes are proportional to haplotype frequencies in the data set. The haplotype detected in the Puerto Real turkey groups within the H3 haplogroup, predominantly observed in modern domestic turkey breeds, historic South Mexican wild turkeys, and modern Rio Grande wild turkey populations.

Highlights

- A turkey coracoid was recovered from a sixteenth-century Spanish site on Hispaniola
- Turkeys are not indigenous to Hispaniola
- Ancient mitochondrial DNA analysis confirms an attribution of *M. gallopavo*
- Biomolecular evidence is consistent with a turkey from Mesoamerica
- Turkeys were part of the post-Columbian merger of diverse cultural traditions