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Can we identify the Mexican hairless dog in the archaeological record?
Morphological and genetic insights from Tizayuca, Basin of Mexico

Aurélie Manin^{a,b,*}, Morgane Ollivier^{c,1}, Fabiola Bastian^{c,2}, Antoine Zazzo^b, Olivier Tombret^b, Juan Carlos Equihua Manrique^d, Christine Lefèvre^b

Affiliation:

a: BioArCh, Department of Archaeology, University of York. Environment building, Wentworth way, YO10 5DD York, United Kingdom

b: Archéozoologie, archéobotanique: Sociétés, pratiques et environnements (UMR 7209), Sorbonne Universités, Muséum national d'histoire naturelle, CNRS, CP55, 55 rue Buffon, 75005 Paris, France

c: CNRS/ENS de Lyon, French National Platform of Paleogenetics, PALGENE, Ecole Normale Supérieure de Lyon, 46 allée d'Italie, 69364 Lyon Cedex 07, France

d: Centro INAH Hidalgo, Calle Casasola S/N, Centro, 42090 Pachuca, Hidalgo, Mexico

1: Present address: UMR CNRS 6553 Ecobio, Université de Rennes 1, Campus de Beaulieu, 35 042 Rennes Cedex, France

2: Present address: Université Claude Bernard Lyon 1, Bât. Gregor Mendel, 43, Bd du 11 novembre 1918, 69622 Villeurbanne Cedex, France.

* corresponding author: Environment Building, Wentworth Way. YO5 10DD York, United Kingdom. aurelie.manin@york.ac.uk

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Abstract: The Mexican Hairless dog, or Xoloitzcuintle, is a breed characterised by a sparse hair coat and a severe oligodontia. This phenotype is a consequence of Canine Ectodermal Dysplasia (CED) caused by a mutation on the *FoxI3* autosomal gene. First accounts of hairless dogs in Mexico are dated to the 16th century CE, according to the historical record, but pre-Hispanic dog skeletons presenting missing and abnormally shaped teeth have been interpreted as earlier evidence of hairless dogs. However, several questions remain unanswered regarding the timing of apparition of this phenotype and its relationship with modern hairless breeds. In this paper, we review the morphological characteristics of potential hairless dogs and we apply ancient mitochondrial DNA analyses along with radiocarbon dating to eight archaeological dog mandibles from Tizayuca, Basin of Mexico, presenting anomalies that could be attributed to a CED. The archaeological dogs were dated between 1620 and 370 years BP. Among these eight individuals, we identify four different mitochondrial haplotypes including two novel haplotypes. The dogs from the Basin of Mexico display a very high genetic diversity and continuity from the Classic to the Postclassic. However, our attempt at amplifying the *FoxI3* mutation was unsuccessful. Finally, we show that some haplotypes are present in both archaeological dogs and modern hairless breeds, perhaps reflecting their maternal ancestry.

Keywords: Mesoamerica; Xoloitzcuintle; Zooarchaeology; Domestication; Ancient DNA; Canine Ectodermal Dysplasia; Radiocarbon Dating;

Highlights

- We studied the mitochondrial DNA from eight ancient Mexican dogs
- The dogs show dental anomalies usually associated with Canine Ectodermal Dysplasia
- Some haplotypes are present in both archaeological dogs and modern hairless breeds
- Mexican dogs display a high genetic diversity and continuity for ca. 1000 years

1. Introduction

While dog (*Canis familiaris*) domestication would have started during the Palaeolithic, breed formation is a recent phenomenon that occurred mostly during the last 200 years (e.g. Larson et al., 2012; Lindblad-Toh et al., 2005; Parker et al., 2017a; Vilà et al., 1997). Some morphotypes were then genetically isolated by reproductive rules to enable the formalisation of breed standards and many breeds that were thought to be of ancient origin (also called primitive breeds) appear to be recent creations (Parker et al., 2004). In Latin America, the Mexican Hairless Dog (also named Xoloitzcuintle) and the Peruvian Hairless Dog are recognised by the World Canine Organisation (FCI) as “Primitive breeds” (<http://www.fci.be>, Group 5 Section 6) although many questions remain unanswered regarding their antiquity (Parker et al., 2017a; van Asch et al., 2013).

American dog populations have been created by two major migration events. The first one occurred about 10,000 years ago when domesticated dogs were first introduced to the continent following human migrations (Leonard et al., 2002; Ní Leathlobhair et al., 2018; Witt et al., 2015). Then, about 500 years ago, European exploration and colonisation of America lead to the introduction of numerous dogs from Western Europe and the progressive vanishing of indigenous maternal lineages (Castroviejo-Fisher et al., 2011). However, some of them may have survived among the modern breeds of American origin (van Asch et al., 2013). Yet, these assertions are mostly based on modern genetic data with limited contribution of archaeological evidence.

The Xoloitzcuintle is one of the Native American breeds with a long-running history. Its first naturalistic description arises from the 16th century (Dibble and Anderson, 1975: 16; Hernández, 1959). At the beginning of the 20th century, hairless dogs are still present in Mexico, although they are uncommon (“The hairless dog,” 1917). The first standard of the breed is established in 1956 from those residual populations (Blanco et al., 2008) and the

Xoloitzcuintle is definitively recognised by the FCI in 1961¹. The breed presents sparse or absent hair coat along with a severe oligodontia and abnormally shaped teeth, both consequences of Canine Ectodermal Dysplasia (CED) of autosomal dominant monogenic inheritance caused by a mutation of the *FoxI3* gene (Drögemüller et al., 2008; Kupczik et al., 2017; Robinson, 1985). Whereas the dental anomalies were not mentioned by the 16th century naturalists, Darwin (2008, p. 58) notices, by the first half of the 19th century, the correlation between the absence of hair and absence of teeth.

Because skin and fur are usually not preserved, archaeologists have used oligodontia and abnormally shaped teeth as an evidence for the presence of hairless dogs in pre-Hispanic sites, along with iconographic depictions of wrinkled and naked dogs (Valadez Azúa et al., 2009; Vásquez Sánchez et al., 2016, 2009). Dogs with missing premolars and teeth of simplified form were identified as hairless dogs and a direct lineage between these and modern hairless breeds has been claimed (Blanco et al., 2008; Valadez Azúa et al., 2013; Vásquez Sánchez et al., 2016) (SI text 1). It is assumed that this morphotype appeared in Western Mexico at the beginning of our era, the first ceramic representations of hairless dogs being found in the region of Colima during the Preclassic (ca. 1500 BCE – 200 CE); from the 6th century CE, they would have spread across Mesoamerica until reaching the Andean region during the 14th century CE (Carot and Hers, 2016; Valadez Azúa et al., 2010, 2009; Vásquez Sánchez et al., 2016) (Figure 1).

¹ <http://www.fci.be/en/nomenclature/XOLOITZCUINTLE-234.html>, consulted on the 21/01/2018.

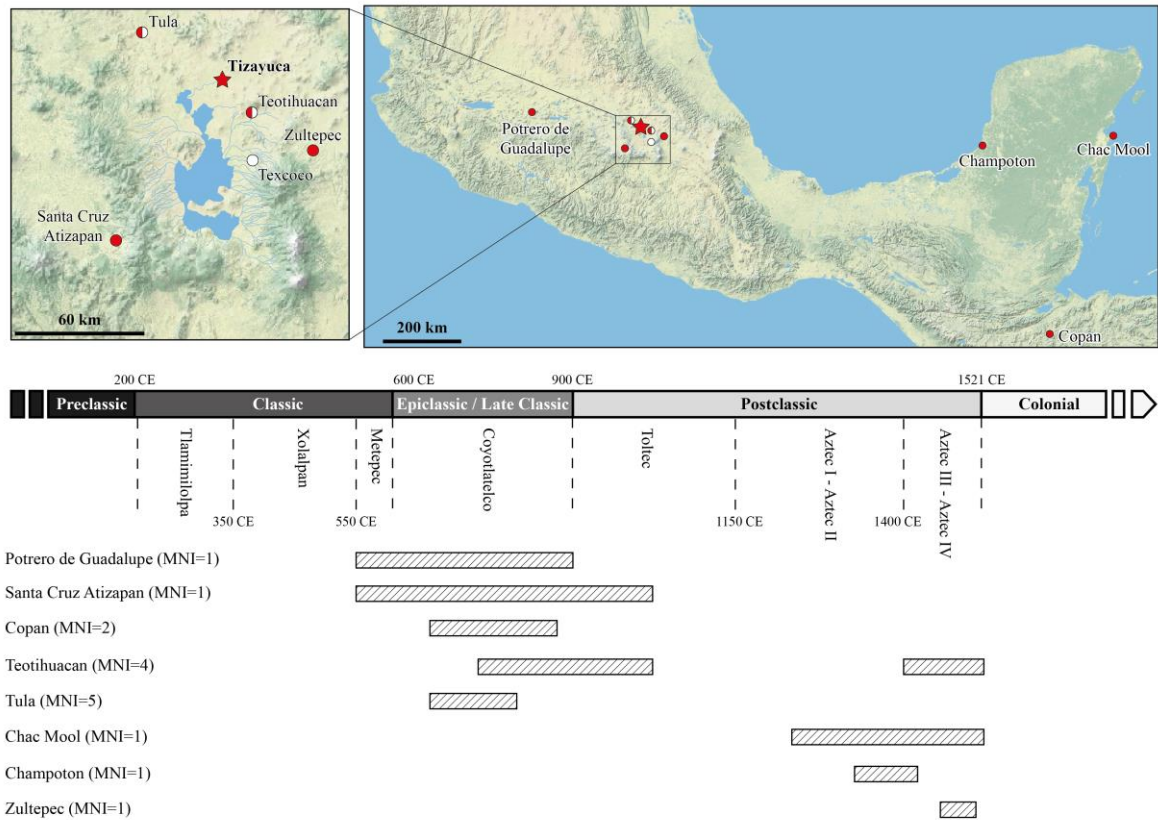


Figure 1 - Top: location of Tizayuca (red star) and other Mesoamerican sites that have yielded remains of dogs identified as hairless (red dots) and aDNA references (white dots). Bottom: chronology of the Basin of Mexico and distribution of the archaeological remains described as hairless dogs in Mesoamerica with the minimum number of individuals (MNI), according to Collins (2002) and Valadez et al. (2009).

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However, no genetic study confirms the relationship between this archaeological morphotype and modern hairless breeds. Indeed, aDNA analyses of archaeological dogs are scarce in Latin America, with only 17 individuals published so far including one possible hairless dog from Tula, Hidalgo (Leonard et al., 2002; Ní Leathlobhair et al., 2018; Valadez et al., 2003) and past genetic diversity remains mainly unknown. Moreover, missing teeth have been observed in several modern dog breeds as well as in wild carnivores (Buchalczyk et al., 1981; Knyazev et al., 2003; Losey et al., 2014; Miles and Grigson, 1990; Szuma, 1999; Vilà et al., 1993), although there is no quantitative estimation of their proportion in the canine population (see SI text 1 for a discussion). Indeed, both a low selective pressure on the number of premolars and a high potential for evolutionary plasticity to diet change on the

molars could contribute to this variability (Asahara, 2013; Vilà et al., 1993). Therefore, the identification of hairless dogs through their phenotype needs to be clarified and further genetic analyses are essential to support affinity between modern hairless breeds and ancient dogs. Finally, accurate dating of each sample is crucial to ascertain their origin and chronological distribution.

Here we apply genetic analyses along with direct radiocarbon dating to eight archaeological dogs from Central Mexico, which show dental anomalies that are similar with modern hairless dogs (referred to here as a “hairless-like” phenotype). Our aims were to clarify the origin of this phenotype and to document the past genetic diversity of dogs from Central Mexico, testing for their relationship with modern breeds, in particular ones of American origin.

2. Material and methods

2.1. The archaeological site of Tizayuca

All the archaeological elements analysed in this study come from three settlements of the *municipio* of Tizayuca, Mexico, excavated between 2006 and 2010 (Equihua Manrique et al., 2008). Architectural, lithic and ceramic elements from Tlamimilolpa, Xolalpan (both associated to the Teotihuacan cultural complex), Toltec and Aztec phases as well as some Colonial remains attest to the persistent occupation of the area from the 3rd to the 16th century CE (Figure 1). Located in the vicinity of the powerful settlements of Teotihuacan, Tula and Mexico-Tenochtitlan, the area of Tizayuca would have been under their direct influence. However, the stratigraphy is highly disturbed and prevented a clear chronological attribution of the ecofacts (Equihua Manrique et al., 2008).

The archaeological area of Tizayuca yielded a total of 3,327 vertebrate remains, from which more than 25% have been attributed to *Canis* sp. (number of identified specimens, NISP = 877). Osteological identification between dog, Mexican wolf (*Canis lupus baileyi*) and coyote (*Canis latrans*) was based on morphological and morphometrical criteria (Blanco Padilla et al., 2009; Lawrence and Bossert, 1967). Measurements were taken following von den Driesch (1976) and ruled out the presence of wild canids in several cases (SI Text 1). We identified at least six juveniles (neonatal to 6 months; Hilson, 2005) and 36 adults (> 7 months; Hilson, 2005), exceeding the presence of any other animal. Canids are followed by leporids (*Sylvilagus* spp., *Lepus* spp., NISP = 271), turkey (*Meleagris gallopavo*, NISP = 144) and large artiodactyls (*Odocoileus virginianus*, *O. hemionus*, cf. *Ovis canadensis*, NISP = 130) (Manin and Lefèvre, 2015). Four dogs (three adults and a juvenile) have been deliberately buried, either as connected or rearranged skeletons, and we also recovered a canine ornament and a tool made from a canid bone. Moreover, burning and butchering marks suggest the consumption of dog meat. These different elements show the importance of dogs to the inhabitants of Tizayuca in both ritual and economic spheres, as was the case in many American cultures (Schwartz, 1997).

2.2. Canid sampling

Among the 36 unpaired canid adult mandibles, several dental anomalies were observed in the premolar row (NISP = 11). The absence of the first premolar (NISP = 3) has been described in many American dog specimens – archaeological and contemporaneous – and is not specifically related to hairless dogs (Allen, 1920: 439; Miles and Grigson, 1990). However, other anomalies have been regularly described as characteristics of hairless dogs (Grouard et al., 2013; Urbano Torrico, 2008; Valadez Azúa, 1995), in particular missing 2nd to 4th premolar and abnormal shape (see criteria in SI Text 1). The eight dog mandibles carrying

177 these anomalies (representing 22% of the dog population in Tizayuca) were selected for
 178 further investigation (Table 1; Figure 2). In order to perform radiometric and molecular
 179 analyses, sections of about 1 cm x 3 cm were opened in the body of each mandible with an
 180 electric saw to take samples for aDNA (0.8 – 2.34 g) and collagen extraction (0.6 – 2.13 g).
 181 To avoid contaminations, the saw and the working space were bleached between each
 182 sampling and a new blade has been used.

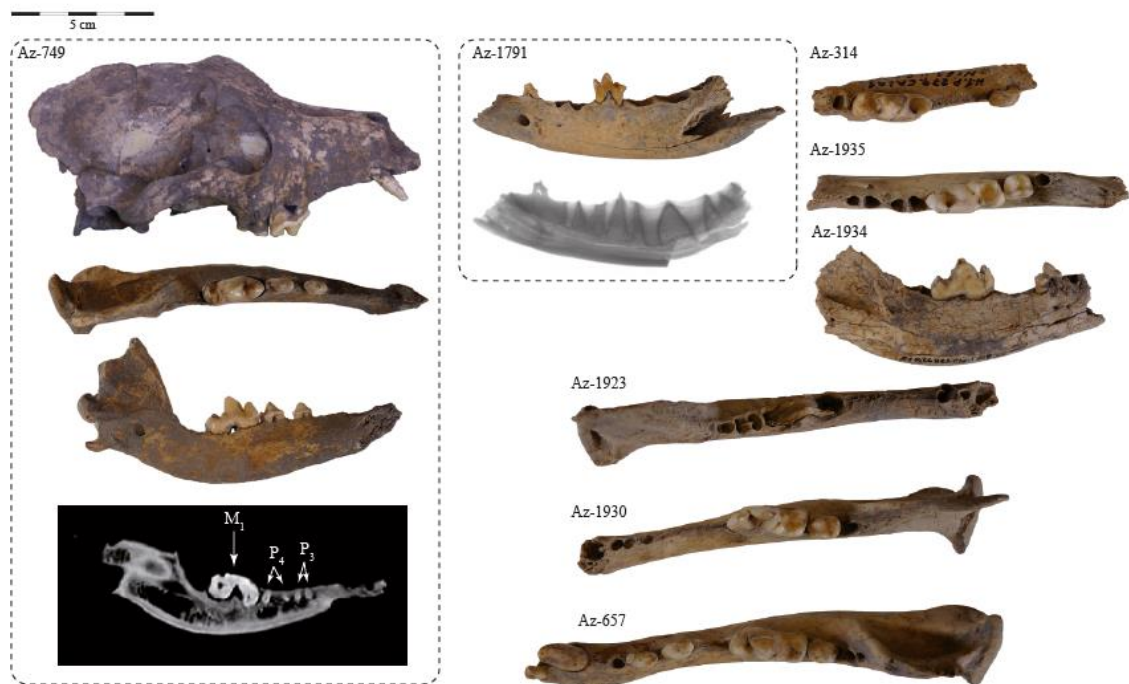


Figure 2 – Illustration of the eight archaeological individuals analysed in this study, including computed tomography (Az-749) and X-ray (Az-1791) images.

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ID	Dental anomalies
Az-314	P ₄ absent
Az-657	P ₄ absent, supernumerary M ₃
Az-749	Only presence of P ₃ , P ₄ , M ₁ ; P ₁ abnormally shaped
Az-1791	Persistence of dp ₂ (or dp ₃) and dp ₄ on adult specimen
Az-1923	P ₃ and P ₄ absent
Az-1930	P ₃ and P ₄ absent
Az-1934	P ₄ absent
Az-1935	P ₂ absent

Table 1: List of dental anomalies observed on the selected mandibles. P = premolar, M = molar, dp = deciduous premolar.

2.3. Radiocarbon dating

Bone collagen from the eight samples was extracted in the Museum national d'Histoire naturelle of Paris (France) and dated using the compact AMS ECHoMICADAS at Gif-sur-Yvette following the method outlined in SI Text 1. All the samples provided acceptable collagen yield [$>1\%$, (Ambrose, 1990)].

2.4. Ancient DNA analyses

All the aDNA extraction and analyses were carried out in the Palgene facility at the Ecole Normale Supérieure of Lyon (France) with tools dedicated to aDNA and strict protocols of decontamination and control. Ancient DNA was extracted following a silica based method (Bastian et al., 2018, see also method outlined in SI Text 1). DNA of the eight samples has been retrieved in two batches consisting of four Mesoamerican dogs, a fish vertebra that was used as cross-contaminant and a blank extraction to rule out aerosol and reagent contaminations.

We used two pairs of published PCR primers (Leonard et al., 2002) to target two overlapping fragments of *Canis* mtDNA control region (CR) producing a 173 bp fragment (position 15515-15687 on the complete mitogenome, Kim et al., 1998). This fragment was chosen as it was short enough to be retrieved even in highly degraded samples, and allows

enough resolution for haplogroup attribution (Frantz et al., 2016), detection of the Latin American clade 1a (Leonard et al., 2002) and individualisation of the American private haplotype A185 (van Asch et al., 2013). Positive amplicons were sequenced on a Ion PGM System (Ion Torrent, Thermofisher).

The sequences were visualised and aligned using Seaview software v.4.6 (Gouy et al., 2010) and the MUSCLE algorithm (Edgar, 2004). Authentic sequences were determined by analysing the reads obtained for each product (218 to 157,669 reads per amplicon) and by considering three independent amplifications for each gene and sample. New SNPs were confirmed by their presence in more than 50% of the reads. Haplotypes were compared through the NCBI BLAST (<https://blast.ncbi.nlm.nih.gov>) to identify matching sequences. Novel haplotypes were described following the recommendations from Pereira et al. (2004). Briefly, the sequences were aligned to the reference genome (Kim et al., 1998) and the position of the polymorphisms was listed unambiguously from this alignment. Position numbers without superscripts denote transitions (e.g., A to G or C to T) whereas other base changes are explicitly indicated.

The sequences produced in this study were then compared to 339 published sequences (Table S1) from American archaeological dogs (Ames et al., 2015; Barta, 2006; Brown et al., 2013; Kemp et al., 2017; Leonard et al., 2002; Thalmann et al., 2013; Witt et al., 2015), American dogs of indigenous origin (Castroviejo-Fisher et al., 2011) selected according to van Asch et al. (2013) and modern dogs from four breeds of presumed American origin: Carolina Dog, Chihuahua, Xoloitzcuintle and Peruvian Hairless Dog (van Asch et al., 2013; Vilà et al., 1999). Multiple alignments of these sequences, number of segregating sites (S), haplotype diversity (Hd), nucleotide diversity (π), Watterson's estimator per sequence (θ_w) and Tajima's D test were assessed using DnaSP v.5.10 (Librado and Rozas, 2009). Analysis of molecular variance (AMOVA) and pairwise F_{ST} population comparison (using the

Reynold's distance and Jukes and Cantor computation of the distance matrix, with a threshold of 0.05) were implemented in Arlequin v.3.5.2.2 (Excoffier et al., 1992; Excoffier and Lischer, 2010). Relationships between the sequences were visualised by constructing phylogenetic trees and median-joining networks (Bandelt et al., 1999).

In order to evaluate past diversity of American dogs, ancient sequences were grouped in six regions according to cultural similarities and geographic proximity: Alaska, North Pacific Coast, Illinois, Florida, American South West, Mesoamerica and South America. Overall archaeological diversity was also compared to modern diversity in the selected sample.

2.5. Modern DNA analyses

In order to amplify the mutation on the first exon of the *FoxI3* gene responsible for the hairless phenotype in modern hairless breeds, we designed a pair of PCR primers targeting a 58-65 bp fragment including the 7 bp duplication previously described (Drögemüller et al., 2008). Modern DNA of different breeds (including hairless and coated ones) was obtained through the Antagene laboratory (France). Amplifications were carried out following the protocol outlined in SI Text 1 §6 and positive amplicons were sequenced on a Ion PGM System (Ion Torrent, Thermofisher). The sequences (0 to 175 reads per amplicon) were visualised and aligned to the first exon of the *FoxI3* gene using MUSCLE (Edgar, 2004) through Seaview software v.4.6 (Gouy et al., 2010). However, none of them matched the targeted sequence (see detailed results in SI Text 1 §6).

3. Results

3.1. Morphological description of the archaeological dogs

From the eight individuals selected for this study (Table 1; Figure 2), seven are isolated mandibles, often fragmented, limiting the reconstitution of the morphotype. The last one (Az-749) is a complete dog that was buried in a Teotihuacan compound (Tlamimilolpa or Xolalpan phase), allowing a more accurate description of the animal.

In Az-749, incisors and canines are missing, but remodelled bone indicates the teeth were lost during the life of the animal. This scar tissue is not present at the location of the P₁, P₂, M₂ and M₃, however, suggesting an agnosis of these dental pieces. The M₁ is present but it displays a reduced talonid. All these anomalies were observed on both left and right mandibles. Computed tomography scanning showed the absence of dental bud inside the mandible. On the skull, only the canine, P⁴ and M¹ are present on both sides but remodelled bone on the dentary indicates the incisors were lost. P¹, P² and P³ would have never erupted, considering the absence of scar tissue. The canines are thin and slightly curved and the P⁴ misses the protocone on both left and right side (Figure 2). Overall, the dog was of medium size, with an estimated shoulder height of 36 to 40 cm (Harcourt, 1974) (Table S2). The presence of a baculum indicates it was a male that died after 6 years according to the pelvis suture (Barone, 1976; Piérard, 1967).

Az-1791 shows the presence of deciduous dp₄ and dp₂ (or dp₃) simultaneously with the alveoli of fully erupted P₃ (or P₄) and M₁. X-ray images indicate the dental buds of the missing permanent premolars never developed (Figure 2). Both Az-1923 and Az-1930 show missing P₃ and P₄ while all the other teeth are present, in particular the P₁ and M₃. On Az-1935, there is no P₂ and despite the breakage pattern it seems P₁ is also missing. P₄ is also missing on Az-657 but two alveolar cavities behind the M₂ suggest an uncommon M₃ with two roots or a supernumerary molar. Finally, Az-314 and Az-1934 show missing P₄ but the fragmentation limits further observations.

3.2. Chronology

272 The radiocarbon dates of the eight samples range from the 4th-6th c. CE to the 15th-17th c.
 273 CE. This age range matches the archaeological occupation of Tizayuca, even for the elements
 274 found in superficial or disturbed layers (Table 2; Figure 3). The oldest sample (Az-749) is
 275 dated from the Classic Period (phase Xolalpan) and is consistent with the context of its
 276 discovery, in an architectural complex strongly influenced by the Teotihuacan culture. Four
 277 specimens cluster in an Epiclassic – Early Postclassic group and three specimens in a Late
 278 Postclassic one. The two more recent samples (Az-1923 and Az-1930) could also pertain to
 279 the Colonial period, albeit with a reduced probability.

Lab ID	Sample	¹⁴ C age (year BP)	Calibrated age (2σ) ^a	Chronological period
ECHo-1528	Az-749	1620 ± 25	386 – 536 CE	Classic
ECHo-1236	Az-657	1170 ± 25	772 – 951 CE	Epiclassic – Early Postclassic
ECHo-1242	Az-1935	1170 ± 25	772 – 951 CE	Epiclassic – Early Postclassic
ECHo-1241	Az-1934	1135 ± 25	777 – 984 CE	Epiclassic – Early Postclassic
ECHo-1235	Az-314	1080 ± 25	895 – 1018 CE	Early postclassic
ECHo-1238	Az-1791	545 ± 25	1318 – 1432 CE	Late Postclassic
ECHo-1240	Az-1930	400 ± 25	1439 – 1619 CE	Late Postclassic – Colonial
ECHo-1239	Az-1923	370 ± 25	1449 – 1631 CE	Late Postclassic – Colonial

Table 2: Results of the radiocarbon dating. ^a Calibrated ages were obtained using the OxCal online program v4.2.4 (Bronk Ramsey, 1994) and the IntCal 13 calibration curve.

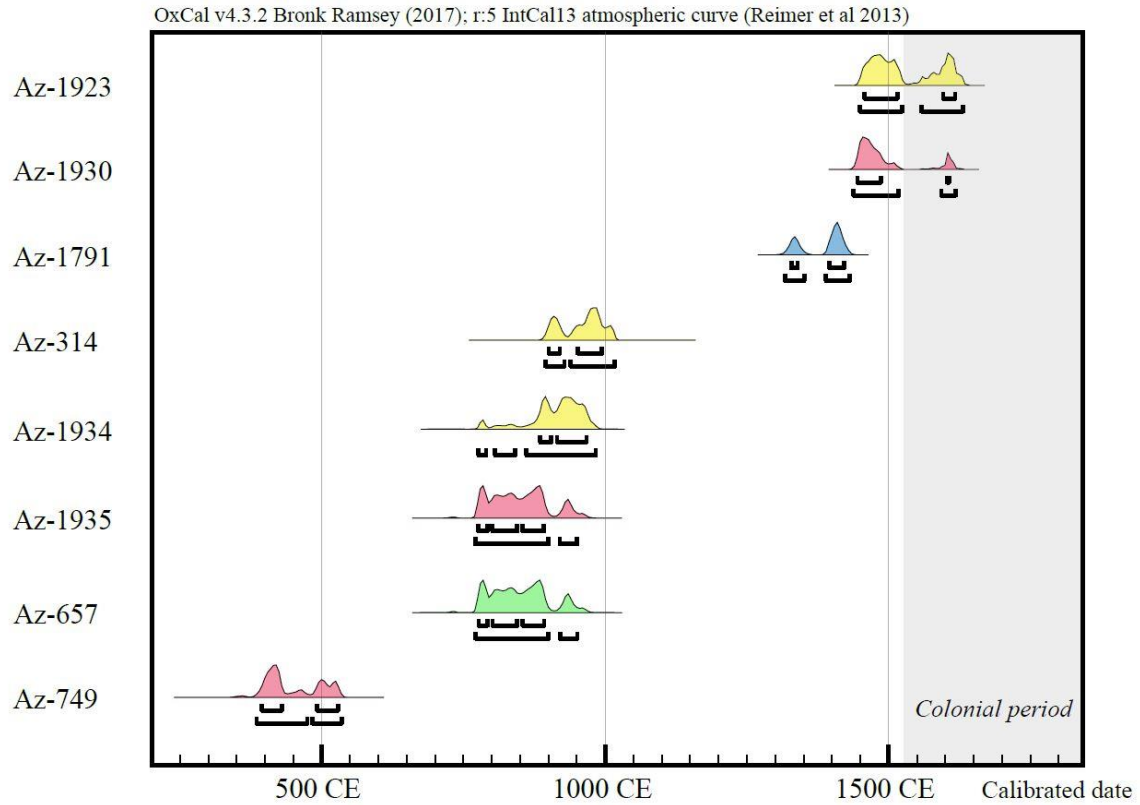


Figure 3: Chronological distribution of the samples with the representation of the haplotypes involved. Age probability is presented with 1 and 2 σ . Calibration was obtained using the OxCal online program v4.2.4 (Bronk Ramsey, 1994) and the IntCal 13 calibration curve. Haplotypes are coloured as follow: red = Xol-H1; green = Xol-H2; Yellow = A11-15-65; blue = A165 (see colours in online version).

Lab ID	Sample	Haplotype	GenBank accession match	Novel haplotype definition
1618	Az-1791	A176	KF002258.1	
1619	Az-1923	A11-15-65	KT321361.1	
1620	Az-1934	A11-15-65	KT321361.1	
1621	Az-1935	(Xol-H1)		15621 15639 ^{T/A} 15651
1622	Az-749	(Xol-H1)		15621 15639 ^{T/A} 15651
1623	Az-1930	(Xol-H1)		15621 15639 ^{T/A} 15651
1624	Az-314	A11-15-65	KT321361.1	
1625	Az-657	(Xol-H2)		15633 15639 ^{T/A}

Table 3 – Results of the mitochondrial aDNA amplification (173 bp fragment of the DLoop). Haplotype names are given after Angleby et al. (2014); when multiple entries were matching identically on GenBank, one only has been given. Novel haplotypes are defined according to Pereira et al. (2004), based on Kim et al. (1998) dog complete mitochondrial genome.

The eight specimens analysed yielded positive amplification of the 173 bp targeted in this study, allowing the identification of four different sequences. Comparison with the NCBI standard nucleotide BLAST for highly similar sequences indicates two corresponding sequences and two novel haplotypes: Xol-H1 and Xol-H2 (Table 3). All the sequences have been submitted to GenBank under accession number MH175494-MH175501.

Three individuals carry a sequence identical to haplotypes A11, A15 and A65 on the targeted 173 bp (Angleby et al., 2014). While A15 and A65 are rare, A11 is a Universal Type (UT) highly frequent in all dog populations (Angleby et al., 2014; Pang et al., 2009; van Asch et al., 2013). One dog carries a sequence identical to haplotype A176, which has only been recognised in a Swedish Dalmatian so far (Angleby et al., 2014), but varies from A11-15-65 of only one mutation on the sequence considered here (T>C, position 15650 on the complete mitogenome, Kim et al., 1998). One novel haplotype (Xol-H1), shared by three archaeological dogs widely separated in time, varies from haplotype A185 (van Asch et al., 2013) by one substitution (C>T, position 15651 on the complete mitogenome, Kim et al., 1998). Haplotype A185 has been considered as a Private Type (PT), only found in the modern Chihuahua breed, and identical sequences have been found in ancient dogs from Central Mexico and North-western America (Ames et al., 2015; Barta, 2006; Leonard et al., 2002). The second novel haplotype (Xol-H2), found in one archaeological dog, differs from A11-15-65 by one substitution (T>C, position 15633 of the complete mitogenome, Kim et al., 1998).

Relationships between these archaeological dogs and other American dogs (both modern and ancient) are illustrated on a median-joining network (Figure 4). The four sequences obtained in this study cluster in Haplogroup A, the most diverse and the most common in ancient American dogs (Thalmann et al., 2013), but none cluster with clade 1a, described by

Leonard et al. (2002) as characteristic from Latin America. Xol-H1 is also closely related with a haplotype carried by modern indigenous dogs from Yucatán (Castroviejo-Fisher et al., 2011) and an ancient Mesoamerican dog from Texcoco (Leonard et al., 2002).

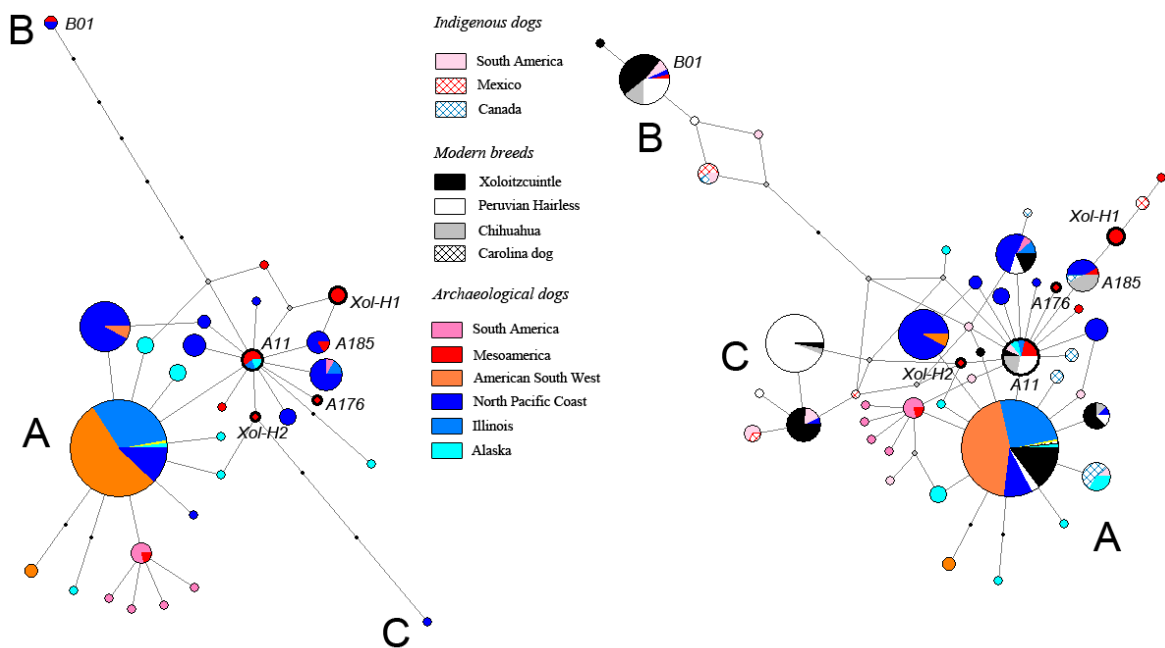


Figure 4 – Median-joining networks displaying the relationships between the obtained sequences (bold circles) and existing archaeological sequences (left; data from Ames et al, 2015; Barta, 2006; Brown et al, 2006; Kemp et al., 2017; Leonard et al., 2002; Witt et al., 2015), with the addition of modern sequences (right; data from van Asch et al., 2013; Castroviejo et al, 2011; Vilà et al. 1999); see colours in online version. Comparative sequences were truncated to the 173 bp analysed in this paper.

3.4. Genetic diversity in the Basin of Mexico

All four haplotypes identified here are different from the five haplotypes previously described in archaeological dogs from the Basin of Mexico (Leonard et al., 2002). No other comparative Mesoamerican samples are available yet, but data from other regions in America enable large-scale comparison of genetic diversity. We used a set of 339 published comparative sequences of ancient and modern dogs (Table 4) to perform diversity tests. All the sequences have been truncated to match the 173 bp fragment targeted in this study.

Most of the published ancient American dogs belong to Haplogroup A, but three individuals belong to Haplogroups B and C. Clade B encompasses one dog from Tula (Mexico), previously identified as a hairless dog (Valadez et al., 2003), and one dog from Gaadu Din Cave (British Columbia, Canada) with no specific dental anomalies reported (Barta, 2006). The last one, in Namu (British Columbia, Canada), pertains to clade C (Barta, 2006).

The genetic variation in archaeological and modern dogs is presented in Table 4. The haplotype diversity H_d is strongly correlated with the nucleotide diversity π ($r = 0.80$, $p = 0.011$) and the Watterson's estimator θ_w ($r = 0.80$, $p = 0.010$), denoting a similar trend between these different indices. However, and although sample sizes are very dissimilar, none of these metrics correlate with the number of individuals in each group ($p > 0.05$), indicating that the diversity observed here is not driven by the sample size and enabling regional comparisons.

Group	n	S	h	H_d (σ)	π (σ)	θ_w (σ)
TOTAL	347	34	46	0.85 (0.02)	0.0192 (0.00094)	5.29 (2.12)
Modern	153	21	25	0.89 (0.01)	0.0255 (0.00095)	3.75 (1.15)
Archaeological	194	30	29	0.71 (0.71)	0.0087 (0.00083)	5.14 (1.43)
Alaska	13	9	8	0.91 (0.06)	0.0114 (0.00205)	2.90 (1.70)
Illinois	34	2	3	0.17 (0.08)	0.0016 (0.00084)	0.49 (0.36)
North Pacific	66	16	11	0.80 (0.04)	0.0116 (0.00137)	3.36 (1.19)
American SW	58	3	3	0.13 (0.06)	0.0012 (0.00057)	0.65 (0.40)
Mesoamerica	13	14	9	0.92 (0.06)	0.0168 (0.00469)	4.51 (2.02)
South America	9	7	6	0.83 (0.13)	0.0090 (0.00283)	2.58 (1.39)

Table 4 – Measure of genetic diversity among the 347 dogs compared in this study, for a 173 bp sequence of the CR: number of individuals (n), number of segregating sites (S), number of different haplotypes (h), haplotype diversity (H_d), nucleotide diversity (π) and Watterson's estimator (θ_w) with associated standard deviation (σ). Mesoamerican dogs (this study, Leonard et al., 2002) are compared to Alaska (Brown, 2006; Leonard et al., 2002), Illinois (Thalmann et al., 2013; Witt et al., 2015), North Pacific Coast (Barta, 2006; Ames et al., 2015), American South West (Kemp et al., 2017; Witt et al., 2015) and South American (Leonard et al., 2002, Thalmann et al., 2013) archaeological populations, and with modern dogs of American origin (indigenous and pure breed, van Asch et al., 2013; Castroviejo et al, 2011; Vilà et al. 1999). One archaeological dog from Florida (Thalmann et al., 2013) is also included in the archaeological group.

Among archaeological samples, those from Mesoamerica show the highest diversity ($H_d = 0.92$; $\pi = 0.0168$; $\theta_w = 4.51$). Conversely, ancient dogs from Illinois (Thalmann et al., 2013; Witt et al., 2015) and from the American South West (Kemp et al., 2017) present an

extremely reduced genetic variability ($H_d = 0.17, 0.13$; $\pi = 0.0016, 0.0012$; $\theta_w = 0.49, 0.65$; respectively).

An AMOVA performed on archaeological dogs from the different regions indicates that the variation within each population accounts for 74 % of the total variation observed in the sample (Table S4). Conversely, the variation between populations only accounts for 26 % of the variation. F_{ST} pairwise population comparison shows the South American dog population is more closely related to the Mesoamerican population than to the other groups (Figure 5, Table S5).

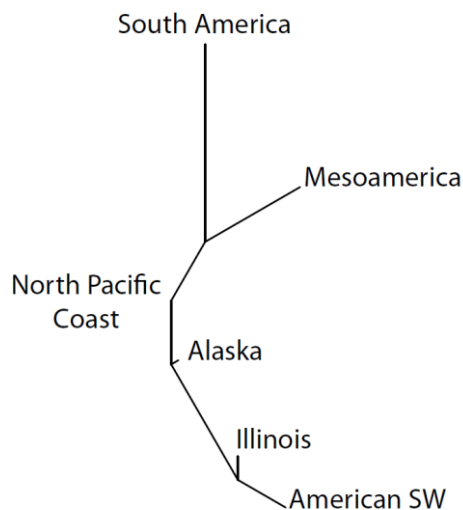


Figure 5 – Unrooted neighbour-joining tree of the F_{ST} pairwise distances showing the relationship between the archaeological populations. Groups follow Table 6.

4. Discussion

4.1. Origin of the “hairless-like” phenotype and presence of hairless dogs in the Teotihuacan cultural area

Direct radiocarbon dating of the eight dogs analysed in this study enable us to clarify their origin. A complete skeleton of hairless-like dog from Tizayuca – Las Golondrinas (Az-749),

dated from the Xolalpan phase (350-550 AD) slightly predates former evidence related to Late Classic / Epiclassic in Western Mexico (Rodríguez Galicia et al., 2001), Central Mexico, (Valadez Azúa and Rodríguez Galicia, 2009) or Honduras (Collins, 2002). The settlement of Las Golondrinas would have had direct contacts with the city of Teotihuacan, visible through the architecture (concrete floors and stucco finishing), funerary practices or lithic and ceramic productions, suggesting it was an enclave of the metropolis (Equihua Manrique et al., 2008).

The presence of hairless dogs in the city of Teotihuacan has also been suggested through the identification of local Classic zoomorphic effigy pots representing dogs with naked skin (Carot and Hers, 2016). Mesoamerican iconography has often been interpreted in naturalistic terms (e.g. de la Garza, 1995; Saunders, 1994; Seler, 1996; Sharpe, 2014; Stocker et al., 1980) and therefore the identification of ancient dog breeds has often been suggested based on ceramic representations (Guzmán and Arroyo Cabrales, 2014). However, no other skeletal remains of “hairless-like” phenotype has been found yet in the Classic occupation of Teotihuacan, despite extensive investigations (Manzanilla and Valadez Azúa, 2009; Valadez Azúa et al., 2013).

4.2. Genetic diversity in the Basin of Mexico

Results from the analysis of a 173 bp fragment of dog CR show the presence of four different haplotypes in Tizayuca, two of them being present in more than one individual. Regarding their chronological repartition (Figure 3), Xol-H1 appears from the Classic to the Late Postclassic / Colonial period. Haplotype A11-15-65 is present from the Epiclassic / Early Postclasssic to the Late Postclassic / Colonial period. Thus, there is an apparent continuity in the dog population from the area of Tizayuca.

Compared to other American regions, the Basin of Mexico displays a high genetic diversity. While the extremely reduced diversity observed in Illinois and the American South

West was interpreted as an expression of bottleneck caused by artificial selection and breeding (Kemp et al., 2017; Witt et al., 2015), the high diversity in the Basin of Mexico could be explained by several factors, including a selection for multiple morphotypes or the presence of a larger and more stable population of dogs. As dental anomalies (including oligodontia) are recognised as inherited characters (Knyazev et al., 2003), their recurrent presence amongst the Mesoamerican dogs suggests regular inbreeding. Indeed, in the site of Tizayuca, we estimate that 22% of the dogs present dental anomalies in the premolar row. In the meantime the diversity of dogs diet in Mesoamerica, as shown by stable isotopes (eg. White et al., 2001), and a greater dental plasticity on the premolar row (Asahara, 2013; Vilà et al., 1993) would allow the variability of dental phenotypes to persist. However, further analyses of full mitogenomes and nuclear markers are required to improve these hypotheses.

Analysis of molecular variance shows there are more differences within the different populations than between them, confirming the low geographic component of domestic dog mtDNA (van Asch et al., 2005; Witt et al., 2015). Interestingly, the neighbour-joining tree built from the F_{ST} pairwise test results indicates clusters differentiating the populations from South America and Mesoamerica from other North American populations (Figure 5). The proximity highlighted between Mesoamerican and South American dogs could be the result of the genetic bottleneck induced by the dispersion of dogs across America, with a loss of genetic variability following the North-South migration. This trend might have been also reinforced by the trade of animals between Mesoamerica and the Andean region. Indeed, eight of the nine South American dogs come from Peru and Bolivia, which is in the heart of the Andean culture. Several archaeological and linguistic evidence point toward continuous, albeit tenuous, exchanges between Mesoamerica and the Andean region during pre-Hispanic times (e.g. Anawalt, 1992; Carot and Hers, 2016; Holser, 1988) and some authors suggested dogs, in particular hairless ones, were part of these movements (Carot and Hers, 2016;

Valadez Azúa et al., 2010, 2009; Vásquez Sánchez et al., 2016). However, further comparative analyses will be necessary to address this question.

4.3. Relationship between archaeological “hairless-like” dogs and modern breeds

The Xoloitzcuintle is considered to be a primitive breed and therefore geneticists have tried to find particular markers differentiating it from other modern breeds (Vilà et al., 1999). Nonetheless recent genomic studies have shown that modern Xoloitzcuintle and Peruvian Hairless dog were strongly influenced by European breeds as a result of recent breed selection, and pre-contact traits would have been highly diluted (Ní Leathlobhair et al., 2018; Parker et al., 2017a). The fact that they share the same causal mutation (Drögemüller et al., 2008) sustains the hypothesis of a common origin but it is not clear if it is due to a pre-contact population movement or modern migrations. Yet, the comparison between these two modern American breeds and archaeological “hairless-like” dogs shows two shared haplotypes: A11, found in three dogs from Tizayuca and B01 found in one dog from Tula (Figure 4). Although we cannot discard their European origin due to post-contact interbreeding, these two haplotypes could reflect the American maternal ancestry of these modern breeds.

The Chihuahua is another breed showing an American origin (van Asch et al., 2013) carrying a PT (A185) already found in pre-contact archaeological samples from Mexico (Leonard et al., 2002) and Northwest America (Ames et al., 2015; Barta, 2006), albeit none of these present a “hairless-like” phenotype. However, novel haplotype Xol-H1, carried by three “hairless-like” dogs from Tizayuca, is closely related with haplotype A185. Interestingly, genomic data show a close relationship between the Chihuahua and the Chinese Crested dog, another hairless breed carrying the *FoxI3* mutation (Parker et al., 2017a). This proximity highlights the complexity of dog history, strengthening the value of aDNA in the analysis of modern breeds and calling for more global studies on the origin of modern breeds.

5. Conclusion

This multidisciplinary analysis of eight dogs with a “hairless-like” phenotype offers new insights into the origin of the American hairless breeds. With direct radiocarbon dating of the samples, we show that “hairless-like” dogs were present in the Basin of Mexico since the Classic, between 386 and 535 CE, and persisted in the archaeological record until 1449 – 1631 CE. It is still unclear, however, if these dogs were the hairless specimens observed by the first Spanish arriving to Mexico. First of all, the dental criteria used to identify them might be associated with other developmental anomalies than CED. Also, other mutations exist that lead to hairless dogs without affecting teeth development (Parker et al., 2017b), stressing the difficulties in accurately identifying them in the archaeological record.

Nonetheless, we show that the dogs from the Basin of Mexico present a high mitochondrial diversity and genetic continuity across the different cultural periods suggesting the existence of a large and stable population of dogs. Some “hairless-like” dogs from Central Mexico present a similar haplotype as some modern Xoloitzcuintli, Peruvian Hairless Dogs and Chihuahua that could reflect one of the maternal ancestries of the breeds.

From the eight specimens analysed, we identified four different haplotypes including two that were never reported before, and all are different from the haplotypes described in ancient Mesoamerican dogs until now. It shows that most of the diversity of ancient American dog populations is still unknown and further studies are required with a larger geographical and chronological sampling to improve our understanding of past and modern dog relationships. Moreover, this study mostly relies on the mtDNA control region whereas complete mitogenome would give a better resolution. On the other hand, mtDNA only represents the maternal half of lineages and plays a limited role in phenotypic expression. Nuclear genomic approaches have been successfully used in ancient dogs to target specific phenotypes (Ollivier et al., 2013), metabolic adaptations (Ollivier et al., 2016), and population history (Botigué et

al., 2017; Ní Leathlobhair et al., 2018; Frantz et al., 2016). Therefore, their application to ancient Mesoamerican dogs would allow for a better understanding of past and modern dog relationships.

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