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1   **Can we identify the Mexican hairless dog in the archaeological record?**

2   **Morphological and genetic insights from Tizayuca, Basin of Mexico**

3

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1

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

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50   **Highlights**

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

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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

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83   Xoloitzcuintle is definitively recognised by the FCI in 1961[1]. The breed presents sparse or

84   absent hair coat along with a severe oligodontia and abnormally shaped teeth, both

85   consequences of Canine Ectodermal Dysplasia (CED) of autosomal dominant monogenic

86   inheritance caused by a mutation of the FoxI3 gene (Drögemüller et al., 2008; Kupczik et al.,

87   2017; Robinson, 1985). Whereas the dental anomalies were not mentioned by the 16th century

88   naturalists, Darwin (2008, p. 58) notices, by the first half of the 19th century, the correlation

89   between the absence of hair and absence of teeth.

90   Because skin and fur are usually not preserved, archaeologists have used oligodontia and

91   abnormally shaped teeth as an evidence for the presence of hairless dogs in pre-Hispanic sites,

92   along with iconographic depictions of wrinkled and naked dogs (Valadez Azúa et al., 2009;

93   Vásquez Sánchez et al., 2016, 2009). Dogs with missing premolars and teeth of simplified

94   form were identified as hairless dogs and a direct lineage between these and modern hairless

95   breeds has been claimed (Blanco et al., 2008; Valadez Azúa et al., 2013; Vásquez Sánchez et

96   al., 2016) (SI text 1). It is assumed that this morphotype appeared in Western Mexico at the

97   beginning of our era, the first ceramic representations of hairless dogs being found in the

98   region of Colima during the Preclassic (ca. 1500 BCE – 200 CE); from the 6th century CE,

99   they would have spread across Mesoamerica until reaching the Andean region during the 14th

100 century CE (Carot and Hers, 2016; Valadez Azúa et al., 2010, 2009; Vásquez Sánchez et al.,

101 2016) (Figure 1).

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[1] http://www.fci.be/en/nomenclature/XOLOITZCUINTLE-234.html, consulted on the 21/01/2018.
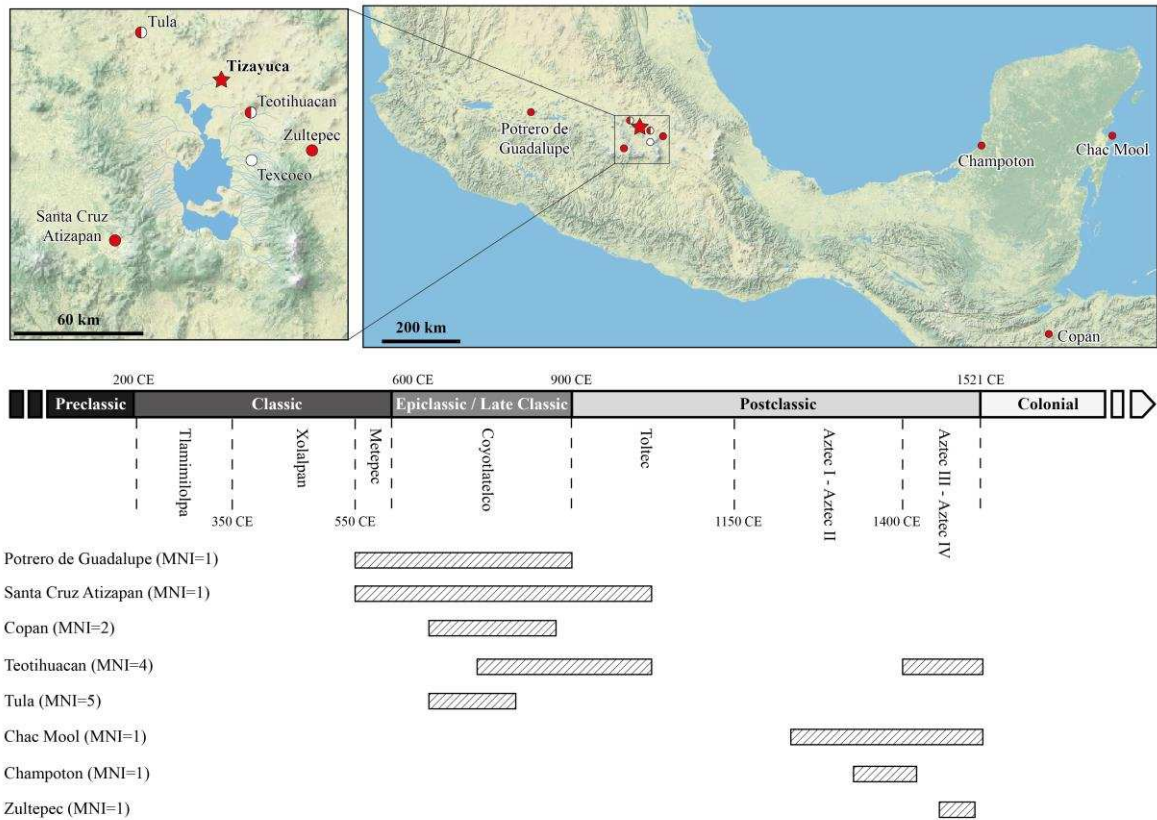
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Figure 1 - Top: location of Tizayuca (red star) and other Mesoamerican sites that have yielded remains of dogs identified as hairless (red dots) and aDNA references (white dots). Bottom: chronology of the Basin of Mexico and distribution of the archaeological 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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114   However, no genetic study confirms the relationship between this archaeological

115   morphotype and modern hairless breeds. Indeed, aDNA analyses of archaeological dogs are

116   scarce in Latin America, with only 17 individuals published so far including one possible

117   hairless dog from Tula, Hidalgo (Leonard et al., 2002; Ní Leathlobhair et al., 2018; Valadez et

118   al., 2003) and past genetic diversity remains mainly unknown. Moreover, missing teeth have

119   been observed in several modern dog breeds as well as in wild carnivores (Buchalczyk et al.,

120   1981; Knyazev et al., 2003; Losey et al., 2014; Miles and Grigson, 1990; Szuma, 1999; Vilà

121   et al., 1993), although there is no quantitative estimation of their proportion in the canine

122   population (see SI text 1 for a discussion). Indeed, both a low selective pressure on the

123   number of premolars and a high potential for evolutionary plasticity to diet change on the

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124   molars could contribute to this variability (Asahara, 2013; Vilà et al., 1993). Therefore, the

125   identification of hairless dogs through their phenotype needs to be clarified and further

126   genetic analyses are essential to support affinity between modern hairless breeds and ancient

127   dogs. Finally, accurate dating of each sample is crucial to ascertain their origin and

128   chronological distribution.

129   Here we apply genetic analyses along with direct radiocarbon dating to eight

130   archaeological dogs from Central Mexico, which show dental anomalies that are similar with

131   modern hairless dogs (referred to here as a "hairless-like" phenotype). Our aims were to

132   clarify the origin of this phenotype and to document the past genetic diversity of dogs from

133   Central Mexico, testing for their relationship with modern breeds, in particular ones of

134   American origin.

135   **2. Material and methods**

136   *2.1. The archaeological site of Tizayuca*

137   All the archaeological elements analysed in this study come from three settlements of the

138   municipio of Tizayuca, Mexico, excavated between 2006 and 2010 (Equihua Manrique et al.,

139   2008). Architectural, lithic and ceramic elements from Tlamimilolpa, Xolalpan (both

140   associated to the Teotihuacan cultural complex), Toltec and Aztec phases as well as some

141   Colonial remains attest to the persistent occupation of the area from the 3rd to the 16th century

142   CE (Figure 1). Located in the vicinity of the powerful settlements of Teotihuacan, Tula and

143   Mexico-Tenochtitlan, the area of Tizayuca would have been under their direct influence.

144   However, the stratigraphy is highly disturbed and prevented a clear chronological attribution

145   of the ecofacts (Equihua Manrique et al., 2008).

146   The archaeological area of Tizayuca yielded a total of 3,327 vertebrate remains, from

147 which more than 25% have been attributed to Canis sp. (number of identified specimens,

148 NISP = 877). Osteological identification between dog, Mexican wolf (Canis lupus baileyi)

149 and coyote (Canis latrans) was based on morphological and morphometrical criteria (Blanco

150 Padilla et al., 2009; Lawrence and Bossert, 1967). Measurements were taken following von

151 den Driesch (1976) and ruled out the presence of wild canids in several cases (SI Text 1). We

152 identified at least six juveniles (neonatal to 6 months; Hilson, 2005) and 36 adults (> 7

153 months; Hilson, 2005), exceeding the presence of any other animal. Canids are followed by

154 leporids (Sylvilagus spp., Lepus spp., NISP = 271), turkey (Meleagris gallopavo, NISP = 144)

155 and large artiodactyls (Odocoileus virginianus, O.hemionus, cf. Ovis canadensis, NISP = 130)

156 (Manin and Lefèvre, 2015). Four dogs (three adults and a juvenile) have been deliberately

157 buried, either as connected or rearranged skeletons, and we also recovered a canine ornament

158 and a tool made from a canid bone. Moreover, burning and butchering marks suggest the

159 consumption of dog meat. These different elements show the importance of dogs to the

160 inhabitants of Tizayuca in both ritual and economic spheres, as was the case in many

161 American cultures (Schwartz, 1997).

162 *2.2. Canid sampling*

163   Among the 36 unpaired canid adult mandibles, several dental anomalies were observed in

164 the premolar row (NISP = 11). The absence of the first premolar (NISP = 3) has been

165 described in many American dog specimens – archaeological and contemporaneous – and is

166 not specifically related to hairless dogs (Allen, 1920: 439; Miles and Grigson, 1990).

167 However, other anomalies have been regularly described as characteristics of hairless dogs

168 (Grouard et al., 2013; Urbano Torrico, 2008; Valadez Azúa, 1995), in particular missing 2nd to

169 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
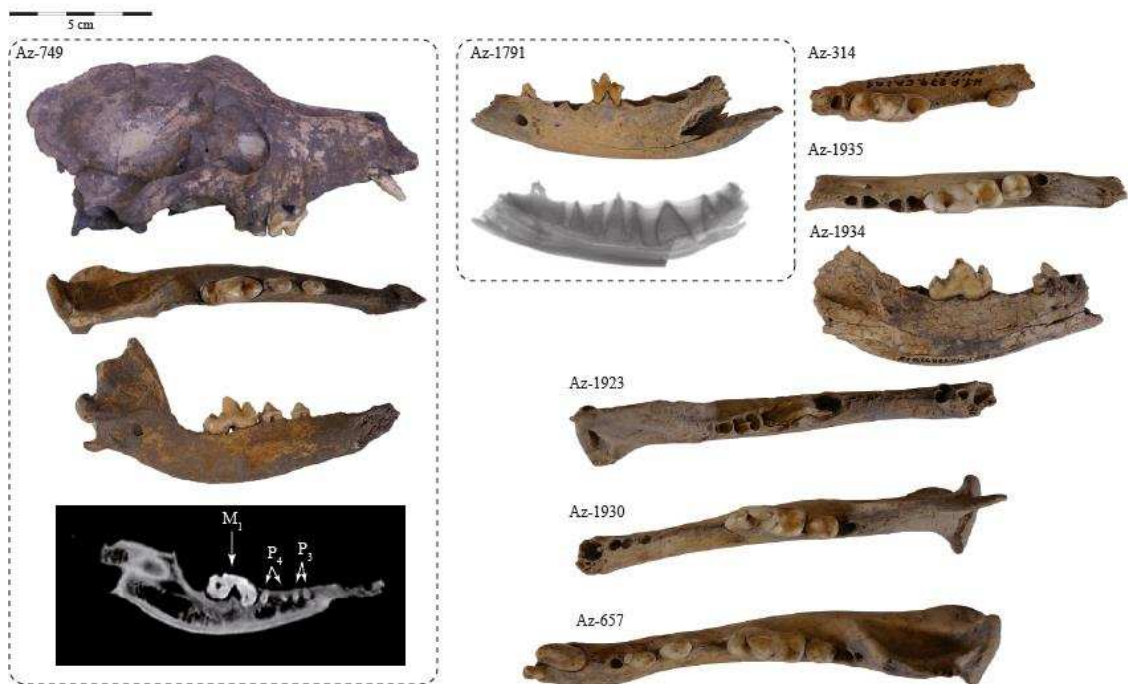
182 sampling and a new blade has been used.

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

183

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

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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

226 Reynold's distance and Jukes and Cantor computation of the distance matrix, with a threshold

227 of 0.05) were implemented in Arlequin v.3.5.2.2 (Excoffier et al., 1992; Excoffier and

228 Lischer, 2010). Relationships between the sequences were visualised by constructing

229 phylogenetic trees and median-joining networks (Bandelt et al., 1999).

230 In order to evaluate past diversity of American dogs, ancient sequences were grouped in

231 six regions according to cultural similarities and geographic proximity: Alaska, North Pacific

232 Coast, Illinois, Florida, American South West, Mesoamerica and South America. Overall

233 archaeological diversity was also compared to modern diversity in the selected sample.

234 *2.5. Modern DNA analyses*

235 In order to amplify the mutation on the first exon of the FoxI3 gene responsible for the

236 hairless phenotype in modern hairless breeds, we designed a pair of PCR primers targeting a

237 58-65 bp fragment including the 7 bp duplication previously described (Drögemüller et al.,

238 2008). Modern DNA of different breeds (including hairless and coated ones) was obtained

239 through the Antagene laboratory (France). Amplifications were carried out following the

240 protocol outlined in SI Text 1 §6 and positive amplicons were sequenced on a Ion PGM

241 System (Ion Torrent, Thermofisher). The sequences (0 to 175 reads per amplicon) were

242 visualised and aligned to the first exon of the FoxI3 gene using MUSCLE (Edgar, 2004)

243 through Seaview software v.4.6 (Gouy et al., 2010). However, none of them matched the

244 targeted sequence (see detailed results in SI Text 1 §6).

245 **3. Results**

246 *3.1. Morphological description of the archaeological dogs*

247 From the eight individuals selected for this study (Table 1; Figure 2), seven are isolated

248 mandibles, often fragmented, limiting the reconstitution of the morphotype. The last one (Az-

249 749) is a complete dog that was buried in a Teotihuacan compound (Tlamimilolpa or

250 Xolalpan phase), allowing a more accurate description of the animal.

251   In Az-749, incisors and canines are missing, but remodelled bone indicates the teeth were

252   lost during the life of the animal. This scar tissue is not present at the location of the P_1, P_2,

253   M_2 and M_3, however, suggesting an agnosis of these dental pieces. The M_1 is present but it

254   displays a reduced talonid. All these anomalies were observed on both left and right

255   mandibles. Computed tomography scanning showed the absence of dental bud inside the

256   mandible. On the skull, only the canine, P^4 and M^1 are present on both sides but remodelled

257   bone on the dentary indicates the incisors were lost. P^1, P^2 and P^3 would have never erupted,

258   considering the absence of scar tissue. The canines are thin and slightly curved and the P^4

259   misses the protocone on both left and right side (Figure 2). Overall, the dog was of medium

260   size, with an estimated shoulder height of 36 to 40 cm (Harcourt, 1974) (Table S2). The

261   presence of a bacculum indicates it was a male that died after 6 years according to the pelvis

262   suture (Barone, 1976; Piérard, 1967).

263   Az-1791 shows the presence of deciduous dp_4 and dp_2 (or dp_3) simultaneously with the

264   alveoli of fully erupted P_3 (or P_4) and M_1. X-ray images indicate the dental buds of the

265   missing permanent premolars never developed (Figure 2). Both Az-1923 and Az-1930 show

266   missing P_3 and P_4 while all the other teeth are present, in particular the P_1 and M_3. On Az-

267   1935, there is no P_2 and despite the breakage pattern it seems P_1 is also missing. P_4 is also

268   missing on Az-657 but two alveolar cavities behind the M_2 suggest an uncommon M_3 with

269   two roots or a supernumerary molar. Finally, Az-314 and Az-1934 show missing P_4 but the

270   fragmentation limits further observations.

271   *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.

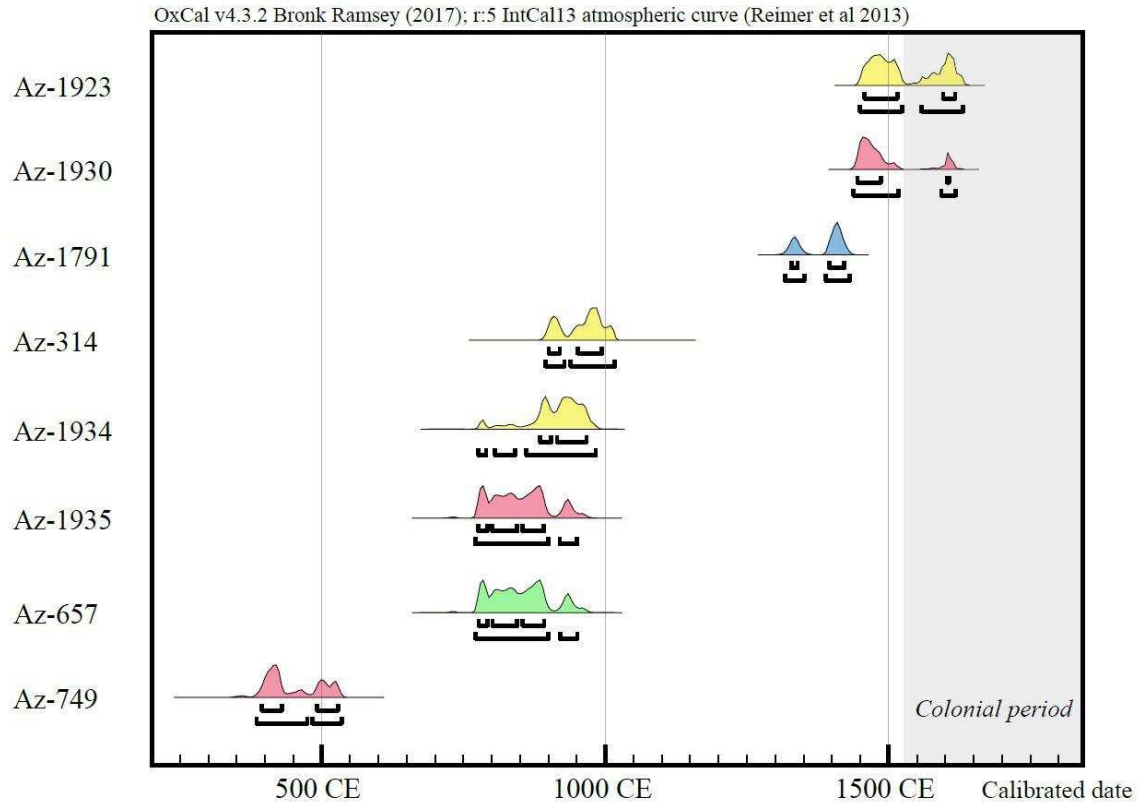OxCal v4.3.2 Bronk Ramsey (2017); r:5 IntCal13 atmospheric curve (Reimer et al 2013)

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.

280   *3.3. Genetic characterisation of the archaeological samples*

281　　　The eight specimens analysed yielded positive amplification of the 173 bp targeted in this

282　　　study, allowing the identification of four different sequences. Comparison with the NCBI

283　　　standard nucleotide BLAST for highly similar sequences indicates two corresponding

284　　　sequences and two novel haplotypes: Xol-H1 and Xol-H2 (Table 3). All the sequences have

285　　　been submitted to GenBank under accession number MH175494-MH175501.

286

287　　　Three individuals carry a sequence identical to haplotypes A11, A15 and A65 on the

288　　　targeted 173 bp (Angleby et al., 2014). While A15 and A65 are rare, A11 is a Universal Type

289　　　(UT) highly frequent in all dog populations (Angleby et al., 2014; Pang et al., 2009; van Asch

290　　　et al., 2013). One dog carries a sequence identical to haplotype A176, which has only been

291　　　recognised in a Swedish Dalmatian so far (Angleby et al., 2014), but varies from A11-15-65

292　　　of only one mutation on the sequence considered here (T>C, position 15650 on the complete

293　　　mitogenome, Kim et al., 1998). One novel haplotype (Xol-H1), shared by three archaeological

294　　　dogs widely separated in time, varies from haplotype A185 (van Asch et al., 2013) by one

295　　　substitution (C>T, position 15651 on the complete mitogenome, Kim et al., 1998). Haplotype

296　　　A185 has been considered as a Private Type (PT), only found in the modern Chihuahua breed,

297　　　and identical sequences have been found in ancient dogs from Central Mexico and North-

298　　　western America (Ames et al., 2015; Barta, 2006; Leonard et al., 2002). The second novel

299　　　haplotype (Xol-H2), found in one archaeological dog, differs from A11-15-65 by one

300　　　substitution (T>C, position 15633 of the complete mitogenome, Kim et al., 1998).

301　　　Relationships between these archaeological dogs and other American dogs (both modern

302　　　and ancient) are illustrated on a median-joining network (Figure 4). The four sequences

303　　　obtained in this study cluster in Haplogroup A, the most diverse and the most common in

304　　　ancient American dogs (Thalmann et al., 2013), but none cluster with clade 1a, described by

305 Leonard et al. (2002) as characteristic from Latin America. Xol-H1 is also closely related with

306 a haplotype carried by modern indigenous dogs from Yucatán (Castroviejo-Fisher et al.,

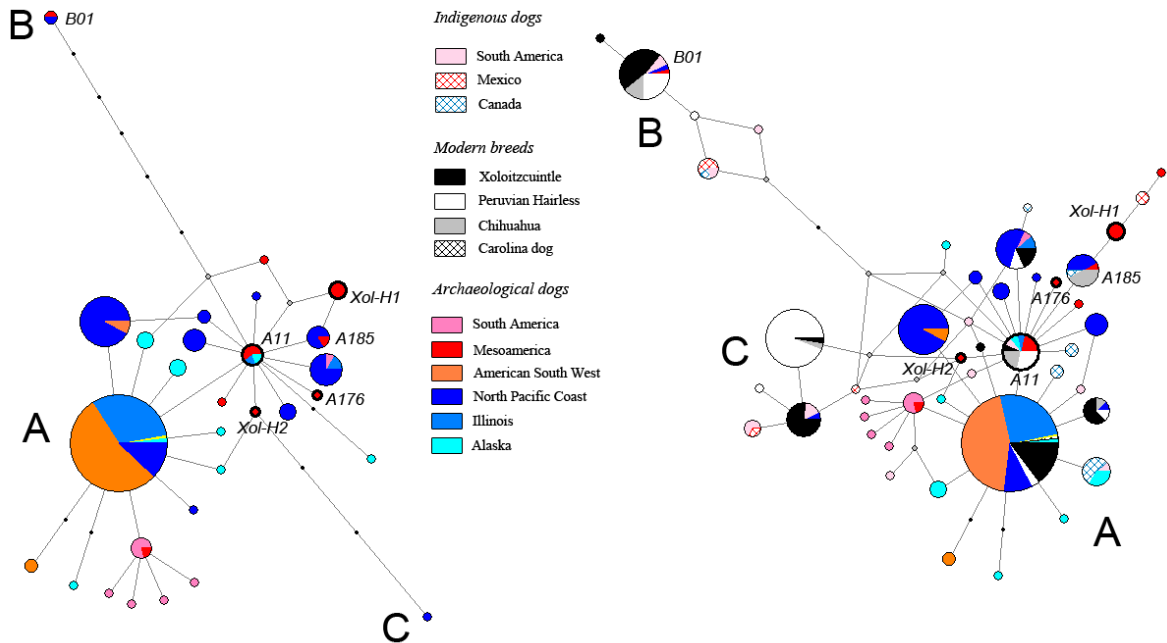307 2011) and an ancient Mesoamerican dog from Texcoco (Leonard et al., 2002).

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.

308   *3.4. Genetic diversity in the Basin of Mexico*

309   All four haplotypes identified here are different from the five haplotypes previously

310 described in archaeological dogs from the Basin of Mexico (Leonard et al., 2002). No other

311 comparative Mesoamerican samples are available yet, but data from other regions in America

312 enable large-scale comparison of genetic diversity. We used a set of 339 published

313 comparative sequences of ancient and modern dogs (Table 4) to perform diversity tests. All

314 the sequences have been truncated to match the 173 bp fragment targeted in this study.

315   Most of the published ancient American dogs belong to Haplogroup A, but three

316   individuals belong to Haplogroups B and C. Clade B encompasses one dog from Tula

317   (Mexico), previously identified as a hairless dog (Valadez et al., 2003), and one dog from

318   Gaadu Din Cave (British Columbia, Canada) with no specific dental anomalies reported

319   (Barta, 2006). The last one, in Namu (British Columbia, Canada), pertains to clade C (Barta,

320   2006).

321   The genetic variation in archaeological and modern dogs is presented in Table 4. The

322   haplotype diversity Hd is strongly correlated with the nucleotid diversity π (r = 0.80, p =

323   0.011) and the Watterson's estimator θ_W (r = 0.80, p = 0.010), denoting a similar trend

324   between these different indices. However, and although sample sizes are very dissimilar, none

325   of these metrics correlate with the number of individuals in each group (p > 0.05), indicating

326   that the diversity observed here in not driven by the sample size and enabling regional

327   comparisons.

Group	n	S	h	Hd (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 (Hd), 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.

328   Among archaeological samples, those from Mesoamerica show the highest diversity (Hd =

329   0.92; π = 0.0168; θ_W = 4.51). Conversely, ancient dogs from Illinois (Thalmann et al., 2013;

330   Witt et al., 2015) and from the American South West (Kemp et al., 2017) present an

331   extremely reduced genetic variability (Hd = 0.17, 0.13; π = 0.0016, 0.0012; θ_W = 0.49, 0.65;

332   respectively).

333   An AMOVA performed on archaeological dogs from the different regions indicates that

334   the variation within each population accounts for 74 % of the total variation observed in the

335   sample (Table S4). Conversely, the variation between populations only accounts for 26 % of

336   the variation. F_{ST} pairwise population comparison shows the South American dog population

337   is more closely related to the Mesoamerican population than to the other groups (Figure 5,
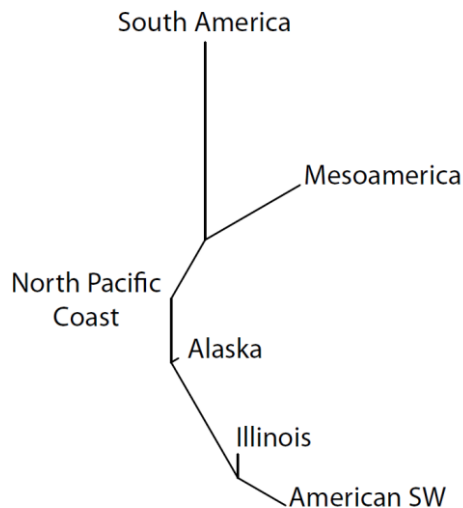
338   Table S5).

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

339   **4. Discussion**

340   *4.1. Origin of the "hairless-like" phenotype and presence of hairless dogs in the*

341   *Teotihuacan cultural area*

342   Direct radiocarbon dating of the eight dogs analysed in this study enable us to clarify their

343   origin. A complete skeleton of hairless-like dog from Tizayuca – Las Golondrinas (Az-749),

344   dated from the Xolalpan phase (350-550 AD) slightly predates former evidence related to

345   Late Classic / Epiclassic in Western Mexico (Rodríguez Galicia et al., 2001), Central Mexico,

346   (Valadez Azúa and Rodríguez Galicia, 2009) or Honduras (Collins, 2002). The settlement of

347   Las Golondrinas would have had direct contacts with the city of Teotihuacan, visible through

348   the architecture (concrete floors and stucco finishing), funerary practices or lithic and ceramic

349   productions, suggesting it was an enclave of the metropolis (Equihua Manrique et al., 2008).

350   The presence of hairless dogs in the city of Teotihuacan has also been suggested through

351   the identification of local Classic zoomorphic effigy pots representing dogs with naked skin

352   (Carot and Hers, 2016). Mesoamerican iconography has often been interpreted in naturalistic

353   terms (e.g. de la Garza, 1995; Saunders, 1994; Seler, 1996; Sharpe, 2014; Stocker et al., 1980)

354   and therefore the identification of ancient dog breeds has often been suggested based on

355   ceramic representations (Guzmán and Arroyo Cabrales, 2014). However, no other skeletal

356   remains of "hairless-like" phenotype has been found yet in the Classic occupation of

357   Teotihuacan, despite extensive investigations (Manzanilla and Valadez Azúa, 2009; Valadez

358   Azúa et al., 2013).

359   *4.2. Genetic diversity in the Basin of Mexico*

360   Results from the analysis of a 173 bp fragment of dog CR show the presence of four

361   different haplotypes in Tizayuca, two of them being present in more than one individual.

362   Regarding their chronological repartition (Figure 3), Xol-H1 appears from the Classic to the

363   Late Postclassic / Colonial period. Haplotype A11-15-65 is present from the Epiclassic / Early

364   Postclasssic to the Late Postclassic / Colonial period. Thus, there is an apparent continuity in

365   the dog population from the area of Tizayuca.

366   Compared to other American regions, the Basin of Mexico displays a high genetic

367   diversity. While the extremely reduced diversity observed in Illinois and the American South

19

368 West was interpreted as an expression of bottleneck caused by artificial selection and

369 breeding (Kemp et al., 2017; Witt et al., 2015), the high diversity in the Basin of Mexico

370 could be explained by several factors, including a selection for multiple morphotypes or the

371 presence of a larger and more stable population of dogs. As dental anomalies (including

372 oligodontia) are recognised as inherited characters (Knyazev et al., 2003), their recurrent

373 presence amongst the Mesoamerican dogs suggests regular inbreeding. Indeed, in the site of

374 Tizayuca, we estimate that 22% of the dogs present dental anomalies in the premolar row. In

375 the meantime the diversity of dogs diet in Mesoamerica, as shown by stable isotopes (eg.

376 White et al., 2001), and a greater dental plasticity on the premolar row (Asahara, 2013; Vilà et

377 al., 1993) would allow the variability of dental phenotypes to persist. However, further

378 analyses of full mitogenomes and nuclear markers are required to improve these hypotheses.

379 Analysis of molecular variance shows there are more differences within the different

380 populations than between them, confirming the low geographic component of domestic dog

381 mtDNA (van Asch et al., 2005; Witt et al., 2015). Interestingly, the neighbour-joining tree

382 built from the F_{ST} pairwise test results indicates clusters differentiating the populations from

383 South America and Mesoamerica from other North American populations (Figure 5). The

384 proximity highlighted between Mesoamerican and South American dogs could be the result of

385 the genetic bottleneck induced by the dispersion of dogs across America, with a loss of

386 genetic variability following the North-South migration. This trend might have been also

387 reinforced by the trade of animals between Mesoamerica and the Andean region. Indeed, eight

388 of the nine South American dogs come from Peru and Bolivia, which is in the heart of the

389 Andean culture. Several archaeological and linguistic evidence point toward continuous,

390 albeit tenuous, exchanges between Mesoamerica and the Andean region during pre-Hispanic

391 times (e.g. Anawalt, 1992; Carot and Hers, 2016; Holser, 1988) and some authors suggested

392 dogs, in particular hairless ones, were part of these mouvements (Carot and Hers, 2016;

393 Valadez Azúa et al., 2010, 2009; Vásquez Sánchez et al., 2016). However, further

394 comparative analyses will be necessary to address this question.

395 *4.3. Relationship between archaeological "hairless-like" dogs and modern breeds*

396 The Xoloitzcuintle is considered to be a primitive breed and therefore geneticists have

397 tried to find particular markers differentiating it from other modern breeds (Vilà et al., 1999).

398 Nonetheless recent genomic studies have shown that modern Xoloitzcuintle and Peruvian

399 Hairless dog were strongly influenced by European breeds as a result of recent breed

400 selection, and pre-contact traits would have been highly diluted (Ní Leathlobhair et al., 2018;

401 Parker et al., 2017a). The fact that they share the same causal mutation (Drögemüller et al.,

402 2008) sustains the hypothesis of a common origin but it is not clear if it is due to a pre-contact

403 population movement or modern migrations. Yet, the comparison between these two modern

404 American breeds and archaeological "hairless-like" dogs shows two shared haplotypes: A11,

405 found in three dogs from Tizayuca and B01 found in one dog from Tula (Figure 4). Although

406 we cannot discard their European origin due to post-contact interbreeding, these two

407 haplotypes could reflect the American maternal ancestry of these modern breeds.

408 The Chihuahua is another breed showing an American origin (van Asch et al., 2013)

409 carrying a PT (A185) already found in pre-contact archaeological samples from Mexico

410 (Leonard et al., 2002) and Northwest America (Ames et al., 2015; Barta, 2006), albeit none of

411 these present a "hairless-like" phenotype. However, novel haplotype Xol-H1, carried by three

412 "hairless-like" dogs from Tizayuca, is closely related with haplotype A185. Interestingly,

413 genomic data show a close relationship between the Chihuahua and the Chinese Crested dog,

414 another hairless breed carrying the FoxI3 mutation (Parker et al., 2017a). This proximity

415 highlights the complexity of dog history, strengthening the value of aDNA in the analysis of

416 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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