DNA analysis of ancient dogs of the Americas: Identifying possible founding haplotypes and reconstructing population histories

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ABSTRACT

As dogs have traveled with humans to every continent, they can potentially serve as an excellent proxy when studying human migration history. Past genetic studies into the origins of Native American dogs have used portions of the hypervariable region (HVR) of mitochondrial DNA (mtDNA) to indicate that prior to European contact the dogs of Native Americans originated in Eurasia. In this study, we summarize past DNA studies of both humans and dogs to discuss their population histories in the Americas. We then sequenced a portion of the mtDNA HVR of 42 pre-Columbian dogs from three sites located in Illinois, coastal British Columbia, and Colorado, and identify four novel dog mtDNA haplotypes. Next, we analyzed a dataset comprised of all available ancient dog sequences from the Americas to infer the pre-Columbian population history of dogs in the Americas. Interestingly, we found low levels of genetic diversity for some populations consistent with the possibility of deliberate breeding practices. Furthermore, we identified multiple putative founding haplotypes in addition to dog haplotypes that closely resemble those of wolves, suggesting admixture with North American wolves or perhaps a second domestication of canids in the Americas. Notably, initial effective population size estimates suggest at least 1000 female dogs likely existed in the Americas at the time of the first known canid burial, and that population size increased gradually over time before stabilizing roughly 1200 years before present.

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Introduction

The domestic dog (Canis lupus familiaris) holds a unique place in the history of animal domestication, in that this species was not only the first to be domesticated, but was also domesticated for a variety of purposes: as guards, hunting aids, and even as companions (Clutton-Brock, 1995). Dog remains dating to 10,000–14,000 years before present (BP) have been discovered across Eurasia, and genetic studies suggest that dogs were domesticated from gray wolves between 11,000 and 20,000 years ago (Germonpré et al., 2009; Pang et al., 2009; Ding et al., 2012; Freedman et al., 2014). Recent analysis of an ancient Siberian canid with a morphology suggestive of a ‘transitional dog’ and a mitochondrial DNA (mtDNA) haplotype found in contemporary dog populations suggests that domestication could have taken place in excess of 33,000 years BP (Druzhkova et al., 2013). The exact origin of domestic dogs is uncertain, though suggested geographic origins include the Middle East, Southeast Asia, and Europe (Pang et al., 2009; Vonholdt et al., 2010; Ardalan et al., 2011; Ding et al., 2012; Thalmann et al., 2013). Most recently, however, results suggest that modern wolf populations diverged from one another at around the same time as dog domestication, and therefore modern populations cannot be used to determine where dogs were first domesticated (Freedman et al., 2014).

Dogs are found in a variety of archaeological contexts in the Americas that date as early as 10,500 years BP, with the first
Dogs have evolved to live with humans, and some of their adaptations provide a historical record of human activity as well. For example, recent studies have examined how genes governing starch digestion differ between dogs and wolves. As dogs were domesticated before the advent of agriculture, it would have been important for them to adapt to the changing human diet and be more efficient at digesting starchy crops. One gene, in particular, which codes for the enzyme alpha-2B-amylase (AMY2B), has two copies in wolves but as many as 30 copies in dogs (Axelsson et al., 2013). These differences in copy number correlate with the history of agriculture in a dog breed’s region of origin. For example, the saluki, which derives from the Fertile Crescent, has 30 copies of the gene while the Siberian husky, which arose in a region with no agriculture, has only two (Freedman et al., 2014). Likewise, human populations have higher copy numbers of salivary amylase (AMY1) in regions with high-starch diets (Perry et al., 2007). Another example can be found in the Tibetan Mastiff, which has adapted to live with humans at high altitudes. A recent study has identified multiple candidate genes for this adaptation (Li et al., 2014), some of which (such as EPAS1, a transcription factor that regulates cell response to hypoxia) are the same genes that have been implicated in human high-altitude adaptation (Xu et al., 2011) and others (such as PLXNA4, a gene that promotes angiogenesis) that share similar functions (Scheinfeldt et al., 2012). Changes in genes expressed in the brain are also commonly found when comparing dogs and wolves (Saetre et al., 2004; Li et al., 2013), suggesting that behavioral differences between wolves and dogs have a genetic basis. These changes in behavior are thought to have arisen early in domestication (Kukkola et al., 2012).

Given the close bond that dogs and humans have shared throughout history, dogs can provide complementary data sources in studies of human populations. Notably, in cases where ancient human remains are inaccessible for use in genetic analysis, dogs can be used as a proxy to examine the population history of humans (Barta, 2006). Critically, organisms that are closely involved with humans have likely moved across the earth following similar routes and at similar times, thus the genetic structure of these populations may reflect upon that of the humans they followed. Like dogs, rats have been distributed worldwide by humans, and they have been used to trace worldwide migration patterns. For example, Polynesian rat populations have been used to inform multiple hypotheses about the origins of humans in Oceania (Matise-Smith and Robins, 2004).

Prior to AD 1492, dogs were widespread across the Americas and have likely been present since humans arrived on the continent (Leonard et al., 2002), so they potentially present an ideal study system for supplementing the reconstruction of human population history in the Americas. Dog remains have been recovered from the Jaguar Cave in Idaho and Agate Basin in Wyoming that date to over 10,000 years BP (Yohhe and Pavesic, 2000). By studying dogs in parallel with humans, we may also learn more about the history of the peopling of the Western Hemisphere than we can by studying humans alone, as dogs can help further test hypotheses about human migration. For example, as dogs have a shorter generation time (four years) than humans (20–30 years) (Fuller, 1995), they have a higher substitution rate than humans, and theoretically they should have accumulated more variation, and possibly genetic structure, over a shorter timescale than possible with human genetic data (Walberg and Clayton, 1981). However, artificial selection (such as breeding) may result in reduced genetic variation or magnified population structure between regional populations.

Using genetic data from contemporary dogs alone to answer questions about the history of pre-contact Native American dogs can be problematic. Genetic studies indicate that much of the diversity of dogs in the early Americas was lost after European contact (Castroviejo-Fisher et al., 2011). A broad sampling of modern village dogs in the Americas demonstrated that nearly all haplotypes identified were shared with dogs introduced to the Americas through European colonization. However, some isolated populations like the Carolina dog and Alaskan Eskimo dog show continuity with ancient samples and retain much of their indigenous diversity, with a maximum of 30% European admixture in the maternal line of extant populations (Van Asch et al., 2013). Due to the collapse of Native American dog populations after AD 1492 and the subsequent replacement of indigenous dog lineages during European colonization, sampling ancient dog remains may provide a much clearer picture of the indigenous population history of dogs and their Native American domesticators in the early Americas.

Humans first entered the Americas roughly 15,000–20,000 years ago (Kemp and Schurr, 2010; Meltzer, 2010). Subsequent movements or expansions into North America from Northeast Asia followed the initial peopling before the Bering Land Bridge became submerged and separated Siberia from the Americas by 10,000–11,000 years ago (Forster et al., 1996; Tamm et al., 2007; Fagundes et al., 2008; Kemp and Schurr, 2010). The initial founders may have followed the Pacific Coast and rapidly spread southward, establishing the Monte Verde site in Chile, for example, approximately 15,000 years ago (Dillehay and Collins, 1988; Schurr and Sherry, 2004; Erlandson, 2007; Tamm et al., 2007; Fagundes et al., 2008). Expansion further inland likely occurred once glaciation withdrew and an ice-free corridor was opened (Hoffecker et al., 1993).

A topic of particular importance in studies of Native American population history is identifying founding mitochondrial haplotypes that were carried by the initial population that populated the Americas. Accurate estimation of the initial diversity found in American populations is key to finding their geographic origin outside of the continents and estimating the founding population size. Torroni et al. (1993a) suggested the use of three criteria in determining which haplotypes of a haplogroup represent founding lineages. First, a founding haplotype is expected to be geographically widespread, cross-cutting linguistic and cultural divisions between Native American populations. Second, the founding haplotype should be central to the phylogeny of the haplogroup, as all other haplotypes in the haplogroup evolved from the founding haplotype, and are thus derived. Lastly, the haplotype should also be found in Siberia or elsewhere in Asia. Initially using these parameters, one founding haplotype was identified in each of the four haplogroups recognized at the time: A, B, C and D (Torroni et al., 1993b). Later, haplogroup X and D4h3a were identified as additional founder lineages using these criteria (Smith et al., 1999; Kemp et al., 2007). Both of these haplogroups have been identified in ancient skeletal remains (Malhi and Smith, 2002; Kemp et al., 2007; Cui et al., 2013; Rasmussen et al., 2014), with D4h3a dating to at least 12,600 years BP. As argued by Kemp et al. (2007), mtDNA types observed in individuals of great antiquity in the Americas are likely to be founding lineages. A unique form of haplogroup M, observed in two ~5000 year old skeletons from the interior of British Columbia (Malhi et al., 2007), while not known to be widespread, may also represent a founder lineage.
More recently, mitogenome data has been used to infer that there were at least 14 founding mitochondrial lineages carried to the Americas: A2, B2, C1b, C1c, C1d, C4c, D1b, D1c, D1d, D2a, D3, D4h3, X2a, and X2g (Tamm et al., 2007; Fagundes et al., 2008; Achilli et al., 2008, 2013; Perego et al., 2009; Malhi et al., 2010; Hooshiar Kashani et al., 2012). Most of these haplogroups are geographically widespread. Notably, each of these haplogroups can be traced to a single ancestral haplotype that is derived by multiple substitutions relative to haplotypes present in Asia, suggesting a period of isolation for the Asia-to-Amerindians migrants from their source population (Tamm et al., 2007; Fagundes et al., 2008). This idea is commonly referred to as the Beringian Incubation Model (BIM) or Beringian Standstill Hypothesis, for which support can also be found in the nuclear genome (Tamm et al., 2007; Schroeder et al., 2009; Villanea et al., 2013).

Estimating the population size of the first humans to enter the Americas is also of particular interest. Changes in the effective population size of Native Americans have been estimated from the initial peopling of the Americas to the present using a Bayesian skyline plot (BSP) analysis incorporating a large number of complete mtgenomes from geographically and linguistically diverse populations (Kitchen et al., 2008; Mulligan et al., 2008). This analysis suggested a three-stage model for colonization, in which there was an initial period of divergence of the migrant population from their Central Asian source population, a period of 7000–15,000 years of population stability in Beringia and a final period of rapid population expansion upon entry into the Americas, with a founder population with an effective size ($N_e$) of 500–1000 females. Critically, this model complements the BIM (Tamm et al., 2007) and suggests that while the initial founding population contained many founder haplogroups that diverged from Asian progenitor haplotypes during the Beringian occupation, the $N_e$ of the founding population of the peopling of the Americas is surprisingly small. Given that the population history of dogs should be similar to that of humans, dogs may show a similarly small effective founding population size.

In this study, we have expanded the sampling of dogs in the early Americas by sequencing individuals recovered from three archaeological sites in North America. Using methods similar to those used for humans, we aim to characterize the population history of dogs in the early Americas by defining founding lineages and examining changes in the dog effective population size over time. In an attempt to move towards such a goal, in this study we sequence a portion of the hypervariable region (HVR) of mtDNA from ancient dog remains from these three archaeological sites. We then combine the sequence data with previously published sequence data from both ancient and modern dogs to identify founding dog mtDNA haplotypes in the Americas as well as infer population history of dogs in the Americas.

**Methods**

**Sampling information and context**

Samples were taken from three distinct archaeological sites across multiple temporal horizons. A map of the approximate location of the archaeological sites from which dogs were previously studied, as well as the location of archaeological sites incorporated in this study can be found in Fig. 1.

**Janey B. Goode (JBG)**

The Janey B. Goode site (11S1232) is a large, prehistoric settlement covering over 6 ha in the American Bottom near Brooklyn, Illinois (Galloy, 2010). The site was occupied approximately 660–1350 years BP, and was most intensively occupied during the Terminal Late Woodland and Mississippian periods. Over 5400 dog remains were recovered, with 103 individual dogs identified (Kuehn, Personal communication). Approximately 80 dog burials were identified, with animals interred individually or in groups of two or three near houses (Borgic and Galloy, 2004). Based on skeletal pathologies, most dogs were used as transport or pack animals. A subset of 39 individuals was identified for genetic analysis, and 35 of those samples were successfully extracted, amplified and sequenced to obtain mtDNA haplotypes.

**Dionisio Point (DP)**

Dionisio Point includes two settlements: a large five-plankhouse village (DgRv-3) that was occupied between 1500 and 1300 years BP and a single plankhouse (DgRv-6) that dates to between 1000 and 700 years BP. Substantial shell middens surround the houses at both sites. Extensive excavations have been completed at both sites over the last two decades (Grier, 2006; Grier et al., 2013) and abundant dog remains have been recovered from both house contexts and midden areas. The sample of eight dogs we analyzed is derived from the shell midden behind the single, later plankhouse at DgRv-6. Dog remains are particularly abundant in this location. Articulated dogs were recovered from various midden layers, both in direct association with human burials and on their own. Isolated or fragmented dog remains were also frequently encountered in the deposits. Dogs of all ages are represented. The association of dog and human burials suggests more than haphazard deposition, and the midden may have been a highly symbolic place. An accurate minimum number of individuals (MNI) for the dogs represented at Dionisio Point has...
not yet been generated; a full analysis of the dog remains is in progress.

Albert Porter Pueblo (APP)

A large Prehispanic Pueblo village in the central Mesa Verde region of southwestern Colorado, Site 5MT123 was occupied intermittently as early as 1400 BP, but most of its occupation dates from the Pueblo II (1100—850 years BP) and Pueblo III (720—850 years BP) periods (Ryan, 2004). The two canid specimens sampled here were excavated between 2001 and 2004 by Crow Canyon Archaeological Center and are part of a larger going-study of dog mtDNA variation in the American Southwest. Architectural details and associated ceramic materials place these specimens within the 940—740 years BP interval (S. Ryan, Personal communication).

DNA extraction and sequencing — University of Illinois Urbana—Champaign (UIUC)

Samples from the Janey B. Goode (JBG) site were extracted in a clean room environment dedicated to the extraction of DNA from ancient organisms at the University of Illinois Urbana—Champaign (UIUC), using a protocol developed previously by the Malhi lab (Cui et al., 2013). No modern dog samples have been processed in the ancient DNA lab, to ensure there is no cross-contamination between modern DNA and the ancient samples. Briefly, all teeth were wiped down with 6% sodium hypochlorite using a Kimwipe at least 1 min to remove surface contaminants, rinsed with molecular grade DNA-free water and dried under UV light, and were drilled using a Dremel drill to produce 0.2 g powder. The powder was then digested in a solution of 4 mL EDTA, 300 μL 10% w/v N-lauryl sarcosine, and 100 μL 3.3% w/v proteinase K. The digestion was concentrated down using a centrifuge to a volume of 250 mL, and then extracted using the QIAQuick PCR Purification kit by Qiagen. All individual samples were extracted at least twice at different times to confirm all DNA sequences. Multiple primers were used to amplify a portion of the hyper-variable region of mtDNA (15421—15691 bp), as listed in Table 1 (Druzhkova et al., 2013). Samples were amplified using the polymerase chain reaction (PCR), with a mix as follows: 2.00 μL DNA, 13.25 μL molecular-grade water, 2.00 μL 10× PCR buffer, 1.20 μL 50 mM MgCl2, 0.80 μL 100 mM dNTPs, 0.30 μL of each primer, concentration 20 mM, and 0.15 μL Platinum Taq DNA Polymerase (Life Technologies). The program used for PCR amplification involved an initial step at 94 °C for 2 min, followed by 40 cycles of 94 °C for 15 s, 55 °C for 15 s, and 72 °C for 12 s, with a final step at 72 °C for 5 min, and successful amplification was verified with gel electrophoresis. Sanger sequencing of the PCR products was performed at the Roy J. Carter Biotechnology Center at UIUC. All individuals were sequenced at least twice for each extraction, and if a consensus was not reached between the extractions, a third extraction was performed to confirm the DNA sequences of individuals. A consensus between two extractions, in which sequences amplified from multiple primers for each of two extractions matched exactly, confirmed the individual’s sequence, and no individual required more than three extractions for sequence confirmation.

DNA extraction and sequencing — Washington State University (WSU)

Eight samples from DP and two samples from APP were extracted in the ancient DNA lab at WSU (Table 2), following the WSU method described in Cui et al. (2013). Samples were first tested for the presence of PCR inhibitors following Kemp et al. (2014) and subjected to repeat silica extraction until they were deemed inhibitor-free.

Two mitochondrial DNA fragments were PCR-amplified using the following primer sets: 1) D15401F (5′-AAGCTCTTGCTCCAC-CATCA-S′) and D15595R (5′-GATATAATATTATGACGTCTGTTAT-S′), 2) D15534F (5′-CTATGTACGTCTCCATGTTATG-S′) and D15711R (5′-GTTGATTTTCTCCAGG-G-S′). Fifteen microliter reactions using Omnilyttag LA were conducted following Kemp et al. (2014) with an annealing temperature of 60 °C. Successful amplification was confirmed via gel electrophoresis and amplicons were prepared and sequenced according to Kemp et al. (2014).

All sequences from this study are available on Genbank (accession numbers KJ189495-KJ189536).

Data analysis

The DNA sequences obtained from the JBG, DP, and APP dogs were combined with other ancient and modern North American dog and wolf mtDNA haplotypes reported in previous studies (Table 3). Ancient DNA haplotypes include samples from 8000 year old dog burials in Siberia, which may have come from the same source population as ancient dogs in the Americas (Losey et al., 2013). Also used were ancient DNA haplotypes from Bolivia, Peru,

Table 1

A list of primers used in sequencing a portion of the hyper-variable region of the mitochondrial genome of the individuals used in the study.

<table>
<thead>
<tr>
<th>Amplicon</th>
<th>Forward</th>
<th>Reverse</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>15421-15617</td>
<td>GCACCCAAACTCTAGATTCT</td>
<td>GACATTAATAGCTCTTGATTAC</td>
<td>This study</td>
</tr>
<tr>
<td>15451-15744</td>
<td>TTTCTGTACACCTCTACATTTAT</td>
<td>GCCCGGACACGGAGAGGGAC</td>
<td>Druzhkova et al., 2013</td>
</tr>
<tr>
<td>15485-15694</td>
<td>CCCCTACGTCTCAGTACATCCTCAG</td>
<td>GCCATGGTATTAAAGCGCTTCAATG</td>
<td>Druzhkova et al., 2013</td>
</tr>
<tr>
<td>15595-15796</td>
<td>CCTTGATAGCAGAGATACTACCTCAG</td>
<td>AGAACAGAGCCAGCGATTAGC</td>
<td>This study</td>
</tr>
<tr>
<td>15608-15863</td>
<td>GCCTAATAGGCGTACATCTGC</td>
<td>TCCAGTGATGTCCCTGAC</td>
<td>Druzhkova et al., 2013</td>
</tr>
<tr>
<td>15776-15982</td>
<td>CTATGCTTGGGCTCCTGATTTGGAC</td>
<td>TTACCAATCGTACGGGGG</td>
<td>This study</td>
</tr>
<tr>
<td>15963-16138</td>
<td>CCGAAGGCGACTACCTAA</td>
<td>TACGGAACCTAACATAT</td>
<td>This study</td>
</tr>
</tbody>
</table>

Table 2

Samples extracted at WSU, indicating amount of each element processed and the number of repeat silica extractions performed.

<table>
<thead>
<tr>
<th>Site</th>
<th>Sample ID</th>
<th>Element</th>
<th>Amount extracted (mg)</th>
<th># Repeat silica</th>
</tr>
</thead>
<tbody>
<tr>
<td>DP</td>
<td>DgRu-6 B1 SF10 PLM</td>
<td>Tooth-P3</td>
<td>28</td>
<td>0</td>
</tr>
<tr>
<td>DP</td>
<td>DgRu-6 B1 SF11 PLM</td>
<td>Tooth-Unerupted M3</td>
<td>35</td>
<td>0</td>
</tr>
<tr>
<td>DP</td>
<td>DgRu-6 B2 West 1 PLM</td>
<td>Tooth-M1</td>
<td>51</td>
<td>0</td>
</tr>
<tr>
<td>DP</td>
<td>DgRu-6 B2 West 2 PLM</td>
<td>Tooth-M2</td>
<td>32</td>
<td>0</td>
</tr>
<tr>
<td>DP</td>
<td>DgRu-6 B2 West 3 PLM</td>
<td>Mandible</td>
<td>55</td>
<td>2</td>
</tr>
<tr>
<td>DP</td>
<td>DgRu-6 B3 East 45</td>
<td>Tooth-P4</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td>DP</td>
<td>DgRu-6 B3 E 58</td>
<td>Tooth-M2</td>
<td>47</td>
<td>0</td>
</tr>
<tr>
<td>DP</td>
<td>DgRu-6 B4 SE 2 PLM</td>
<td>Tooth-M2</td>
<td>31</td>
<td>0</td>
</tr>
<tr>
<td>APP</td>
<td>5MT123 PD 1305 FS42</td>
<td>Tooth-Unknown</td>
<td>54</td>
<td>0</td>
</tr>
<tr>
<td>APP</td>
<td>5MT123 PD 1936 FS2</td>
<td>Tooth-Incisor</td>
<td>42</td>
<td>1</td>
</tr>
</tbody>
</table>

Dionisio Point is abbreviated DP and Albert Porter Pueblo as APP.
Mexico, Argentina, western Canada, and the United States (Koop et al., 2000; Leonard et al., 2002; Brown et al., 2013; Byrd et al., 2013; Thalmann et al., 2013). Modern haplotypes of North American and Eurasian wolves and all published dog haplotypes were also used for comparison (Tsuda et al., 1997; Vila et al., 1997; Okumura et al., 1999; Savolainen et al., 2002; Takahasi and Miyahara, 2002; Pang et al., 2009; Klütsch and de Caprona, 2010; Castroviejo-Fisher et al., 2011; Thalmann et al., 2013; Van Asch et al., 2013). The DNA sequences obtained from the literature varied in length, so sequences were trimmed to produce a dataset that maximizes the number of individuals incorporated while showing variation along a shorter segment of 229 base pairs [nucleotide positions (nps) 15458–15687, according to Genbank Accession NC002008] of the mitochondrial genome.

Putative founding haplotypes were identified using the following criteria, modeled after similar criteria used for inferring founding haplotypes of Native Americans (Torroni et al., 1993a). A founding haplotype should be present in multiple geographic regions of the Americas and should be central to a phylogeny of dog mtDNA sequences. Additionally, a founding haplotype may be found both in the Americas and in Asia. However, if a haplotype is found to be infrequent or geographically localized in the Americas and also differs by multiple substitutions from other dog haplotypes, it may also be a putative founding haplotype. It would be more likely for a sequence that differs by five or more substitutions from other founding haplotypes to be another founding haplotype than for it to be derived from a much more distant haplotype.

The dataset of ancient dog and modern dog and wolf sequences was aligned using BioedIt, and the program Network was used to construct a median-joining network for the DNA sequences (Bandelt et al., 1999). A network is a visual representation of how haplotypes relate to one another and is useful to determine if certain haplotypes are shared among populations. The median-joining method constructs trees that minimize the genetic distance between haplotypes by linking clusters of closely-related sequences, and then resamples those clusters to produce the most parsimonious network (Bandelt et al., 1999). For each geographic region studied as well as for the wolf samples, additional calculations were made using Arlequin (Excoffier and Lischer, 2010) to compare diversity within and between groups. For each group, the number of haplotypes and the number of segregating sites in the sample corrected by the number of individuals in a sample (Watterson, 1975), were calculated for each group. Nucleotide diversity was calculated for each group as a measure of sequence diversity weighted both by haplotype frequency and the number of substitutions that differ from other haplotypes in the group. These measures of genetic diversity are often used to compare populations and estimate how populations differ from one another. A population with lower diversity might indicate a bottleneck event or deliberate breeding, and a population with higher diversity might indicate a larger and more stable population size over a longer period of time or a population that experienced gene flow. Since only two samples were analyzed from the Albert Porter Pueblo population, they were omitted from this analysis. In some cases, such as in Alaska and Mexico, samples are derived from multiple archaeological sites, in which case measures of diversity were also calculated for each archaeological site containing multiple dogs. Finally, an analysis of molecular variance (AMOVA) was also performed to estimate groupings that explained the most variation, to estimate what populations were most closely related.

Some mtDNA haplotypes were identified as ‘outliers’. These outliers were ancient dog haplotypes that differed from other ancient dog haplotypes by at least four substitutions, and are either haplotypes shared with wolves or putative founding haplotypes due to their genetic distance from the other sequences. In addition to what was reported in the literature, these haplotypes were identified as wolf or dog haplotypes by constructing a distance tree. Using all modern dog haplotypes, all published wolf haplotypes and all ancient dog haplotypes, a phylogeny was constructed in MEGA, using a maximum likelihood criterion with an HKY + I + G model of substitution, with estimated substitution rates (Bandelt et al., 1999). A network is a visual representation of how haplotypes relate to one another. A population with lower diversity might indicate a bottleneck event or deliberate breeding, and a population with higher diversity might indicate a larger and more stable population size over a longer period of time or a population that experienced gene flow. Since only two samples were analyzed from the Albert Porter Pueblo population, they were omitted from this analysis. In some cases, such as in Alaska and Mexico, samples are derived from multiple archaeological sites, in which case measures of diversity were also calculated for each archaeological site containing multiple dogs. Finally, an analysis of molecular variance (AMOVA) was also performed to estimate groupings that explained the most variation, to estimate what populations were most closely related.

A dataset consisting of ancient dog haplotype sequences not closely related to wolf haplotypes (i.e., those not likely of wolf-dog admixture) and all modern dog haplotype sequences found in dog breeds originating in the Americas was analyzed under a Bayesian coalescent framework. This analysis was performed using the Bayesian Markov chain Monte Carlo (MCMC) methods implemented in BEAST (version 1.8.0; Drummond et al., 2012). For this analysis, an HKY + G + I substitution model, a strict molecular clock, and an extended Bayesian skyline plot (EBSP) demographic model (with linear changes in population size; Heled and Drummond, 2008) was used to infer the historical dynamics of dog populations. Importantly, the family of Bayesian skyline plot demographic models provides a means to infer past population histories using both ancient and contemporary genetic samples without a priori definition of a parametric model of past population dynamics (i.e., constant or exponentially growing population size). Mean dates for archaeological sites were used as sampling dates (Table 4 and Supplementary Online Material [SOM] Table 1) of the aDNA sequences and provided independent calibrations for the molecular clock; a CMTC prior was used as a prior for the substitution rate (Ferreira and Suchard, 2008). Unless otherwise noted, default priors and operator values were used. Markov chains were run for 200 million generations with samples taken every 10,000 generations; convergence of three independent MCMC runs was assessed using Tracer (version 1.6; Rambaut and Drummond, 2007), and all MCMC samples were combined after the first 10% of samples were discarded as burn-in. The combined data files were used for final inferences about the historical population dynamics of Native American dogs and to produce a summary genealogy of the HVRI sequences in this dataset.

To assess the accuracy of our coalescent inference and determine if our punctuated sampling of ancient dog HVRI sequences introduced a bias to our analyses, two sets of simulations were performed. First, ten subsets of the dataset consisting of a random sample of 25 aDNA sequences across all times were also analyzed in BEAST using the same models and priors from the analysis of the full dataset. This was done to assess any possible bias in the

| Table 3 |

A list of mtDNA data of ancient dogs in the Americas in the literature.

<table>
<thead>
<tr>
<th>Origin</th>
<th>Age (years BP)</th>
<th># Individuals</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Siberia</td>
<td>8000</td>
<td>4</td>
<td>Looey et al., 2013</td>
</tr>
<tr>
<td>Bolivia</td>
<td>&gt;1000</td>
<td>5</td>
<td>Leonard et al., 2002</td>
</tr>
<tr>
<td>Peru</td>
<td>1000</td>
<td>3</td>
<td>Leonard et al., 2002</td>
</tr>
<tr>
<td>Argentina</td>
<td>1000</td>
<td>1</td>
<td>Thalmann et al., 2013</td>
</tr>
<tr>
<td>Mexico</td>
<td>1400–800</td>
<td>5</td>
<td>Leonard et al., 2002</td>
</tr>
<tr>
<td>Alaska</td>
<td>–400–600</td>
<td>11</td>
<td>Leonard et al., 2002</td>
</tr>
<tr>
<td>Alaska</td>
<td>–200–800</td>
<td>7</td>
<td>Brown et al., 2013</td>
</tr>
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<td>California</td>
<td>–900–400</td>
<td>3</td>
<td>Byrd et al., 2013</td>
</tr>
<tr>
<td>Koster, Illinois</td>
<td>9000</td>
<td>1</td>
<td>Thalmann et al., 2013</td>
</tr>
<tr>
<td>Florida</td>
<td>1000</td>
<td>1</td>
<td>Thalmann et al., 2013</td>
</tr>
<tr>
<td>Western Canada</td>
<td>Unknown</td>
<td>5</td>
<td>Koop et al., 2000</td>
</tr>
</tbody>
</table>

The general location of the archaeological site(s) is included, as well as the date (if the samples were dated) and the number of individuals.
estimation of past population dynamics introduced by both non-random space (i.e., the inter-relatedness of dogs at sites) and time (i.e., multiple samples come from the same horizon). Second, we performed simulations to produce synthetic datasets with a known constant demographic history and substitution and clock model parameters identical to those estimated in the analysis of the full dataset. These simulations were performed in BEAST (Bielejec et al., 2011) and analyzed with the HKY + G + 1 substitution, strict clock, and EBSP demographic (linear variant) models. One hundred simulations were performed to assess whether punctuated sampling of low information genetic data might produce artifacts in reconstructed skyline plots.

To compare shifts in dog population size with shifts in human population size, an EBSP was constructed using the dataset employed in O’Fallon and Fehren-Schmitz (2011), which consists of living individuals representing all five Native American haplogroups and ancient individuals from Ontario, Illinois, and Peru, and is thus similar to our dataset in having both modern and ancient sequences. To allow for direct comparison, only the HVRI of these individuals was used in the analysis. This analysis was also performed in BEAST 1.8.0 using an HKY + G + 1 substitution model, an uncorrelated log-normally distributed relaxed molecular clock (Drummond et al., 2006) with a fixed mean substitution rate of 1.64 × 10^{-7} substitutions/site/year (Soares et al., 2009) and an EBSP demographic model. Sampling dates for the ancient individuals were taken from the original analysis (O’Fallon and Fehren-Schmitz, 2011). To assess the accuracy of demographic reconstruction using short HVRI sequences, 100 simulations were performed in which synthetic datasets were produced from known demographic history and analyzed in BEAST. Synthetic data were simulated using the same substitution model parameters estimated in the empirical analysis and under a demographic history similar to that inferred from the empirical data: an initial population size of 5^3 at 15,000 years BP, and a sudden decrease in population size to 5 × 10^4 years BP. These synthetic datasets were analyzed with the HKY + G + 1 substitution, strict clock (rate = 1.64 × 10^{-7} substitutions/site/year), and EBSP demographic (linear variant) models. All graphs were produced using R.

Results

The individuals sequenced in this study represent a total of nine different HVRI haplotypes, four of which are novel. Novel haplotypes were authenticated by confirming identical sequences from two extractions, as well as by amplifying and sequencing each extract twice, to ensure that the novel substitutions are not due to miscoding lesions, a common problem in ancient DNA analysis (Gilbert et al., 2003). All haplotypes are shown in SOM Table 1. Due to the short length of the sequence, nearly all of the individuals with haplotypes that are not novel have identical sequences to multiple modern dog haplotypes. The measures of genetic diversity are shown in Table 4 for all geographical regions, and in Table 5 are subdivided further by archaeological site. Taking a regional perspective, Alaska and Mexico have the highest diversity, followed by California and western Canada. Siberia, Bolivia, Illinois (represented by JBG) and coastal British Columbia (represented by DP) have much lower levels of diversity. When subdivided into archaeological sites, Tula, Mexico, has the highest diversity,

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Measures of genetic diversity of ancient dog populations by region.</th>
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<tbody>
<tr>
<td><strong>Population</strong></td>
<td><strong># Individuals</strong></td>
</tr>
<tr>
<td>Janey B. Goode</td>
<td>34</td>
</tr>
<tr>
<td>Dionisio Point</td>
<td>5</td>
</tr>
<tr>
<td>Siberia</td>
<td>4</td>
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<tr>
<td>Alaska</td>
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<td>Mexico</td>
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<td>Boliviana</td>
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<tr>
<td>Peru</td>
<td>3</td>
</tr>
<tr>
<td>Wolf</td>
<td>7</td>
</tr>
<tr>
<td>Dogs</td>
<td>83</td>
</tr>
</tbody>
</table>

For each population, the number of individuals and haplotypes is recorded, as well as Theta S, a measure of the number of segregating sites per nucleotide weighted by sample size. Nucleotide diversity is also included, a measure of diversity that incorporates pairwise comparisons between samples, and was calculated both with equal haplotype frequencies and with weighted haplotype frequencies.

Table 5

<table>
<thead>
<tr>
<th>Table 5</th>
<th>Measures of genetic diversity of ancient dog populations by archaeological site.</th>
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</thead>
<tbody>
<tr>
<td><strong>Population</strong></td>
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<td>Fairbanks, AK</td>
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<tr>
<td>W. Alaska</td>
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<tr>
<td>Tula, Mexico</td>
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<tr>
<td>California</td>
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<tr>
<td>Western Canada</td>
<td>5</td>
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</tbody>
</table>

For each population, the number of individuals and haplotypes is recorded, as well as Theta S, a measure of the number of segregating sites per nucleotide weighted by sample size. Nucleotide diversity is also included, a measure of diversity that incorporates pairwise comparisons between samples, and was calculated both with equal haplotype frequencies and with weighted haplotype frequencies.
followed by western Alaska and California; the diversity at the Fairbanks, Alaska site is much lower.

The network in Fig. 2A shows all published ancient dogs of the Americas compared with wolf haplotypes worldwide. A network representing all published ancient dog haplotypes from the Americas prior to this study can be found in SOM Fig. 1. The addition of samples from Janey B. Goode, Dionisio Point and Albert Porter Pueblo introduces six new mitochondrial haplotypes not found previously in dogs from the Americas. One haplotype is found to have a particularly high frequency in the sample, and is represented by 28 dogs from Janey B. Goode, as well as one dog each from Illinois, Florida, California, Albert Porter Pueblo, and Siberia as well as one Asian wolf (a similar network color-coded by geographic region is presented in SOM Fig. 2). Notably, many of the other ancient dog haplotypes differ from this common haplotype by only one or two nucleotide substitutions. There is also some regional clustering of haplotypes from ancient dogs — nearly all haplotypes from South American dogs cluster closely together and differ only by a few substitutions, whilst most of the Janey B. Goode haplotypes only differ by a few substitutions as well. Interestingly, there are also a few clusters of wolves, highlighting the distinctiveness of the wolf versus dog haplotypes. However, some dog haplotypes are nearly identical to wolf haplotypes, and are separated by only one or two substitutions. Specifically, most haplotypes from the Canadian dog sample are shared with or only slightly distant from a North American wolf haplotype, whilst a Mexican dog haplotype is

Figure 2. Mitochondrial haplotype networks of ancient dogs in the Americas. Arctic dogs are considered to be individuals from archaeological sites in western Canada and Alaska. Each color node represents a haplotype, a unique mitochondrial DNA sequence. The size indicates the number of individuals with the particular haplotype. The length of the lines connecting the nodes indicate the number of substitutions that separate any two haplotypes. Small red diamonds indicate uncertainty: in these cases it is impossible to tell the exact order of the substitutions in question. (A) Network of ancient American dog haplotypes with ancient and modern wolf haplotypes, including those published in this study. (B) Network of ancient American dog haplotypes, including those published in this study. Asterisks by a haplotype indicate that it is a putative founding haplotype. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

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identical to a European wolf haplotype that differs by only a single substitution from a North American wolf haplotype.

A second network was constructed that consists solely of ancient dog samples, to provide an illustration of how the dog haplotypes relate to one another without incorporating the wolf haplotypes, and is represented by Fig. 2B (a similar network color-coded by geographic region is presented in SOM Fig. 3). It is more apparent here that there are multiple outlier haplotypes. As discussed above, four of the western Canadian dogs and the Mexican dog in the figure are likely the result of admixture between dogs and wolves. These wolf-like lineages may indicate that dogs were separately domesticated in the Americas. A single haplotype represented by an Alaskan dog seems dissimilar to both dogs and wolves on the network in Fig. 2A, but all individuals with more than four substitutions different from other dog haplotypes in Fig. 2B are considered to be outliers.

A phylogeny was constructed to infer the relationship of the outlier sequences to all published dog and wolf haplotypes (Fig. 3). Overall, the wolf and dog haplotypes seem to be mixed throughout the tree. The haplotypes from the ancient dogs that are identical to a European wolf haplotype that differs by only a single substitution from a North American wolf haplotype, (ii) haplotypes that show admixture with North American wolves, or possibly (iii) the result of a separate domestication of dogs in the Americas. Notably, multiple outlier haplotypes are more closely related to wolves than dogs, suggesting admixture or independent domestication. One haplotype from an Alaskan dog is not closely related to modern dog or wolf haplotypes, whilst two other outlier haplotypes cluster more closely with modern dog haplotypes than wolf haplotypes. Interestingly, the latter two outliers cluster with some haplotypes that are only found in Siberia, the Americas or eastern Asia (Pang et al., 2009), possibly indicative of a Northeast Asian origin, consistent with the origin of Native Americans (Forster et al., 1996).

The EBSP of the dogs shows a relatively stable population from the present to the time of the most recent common ancestor, ~9000 years BP, with the exception of a small dip in population size around 1000 years BP (Fig. 4A). A summary genealogy relating the dog samples in the dataset produced from the posterior distribution of genealogies sampled in the Bayesian coalescent analysis is presented in SOM Fig. 5. The simulations using random subsets of the ancient samples show no decrease in effective population size over time, suggesting that the population decline is likely an artifact of...
the sampling, as shown in the composite EBSPs in SOM Fig. 1. Furthermore, the simulations in which synthetic datasets produced under a constant population size model and identical sampling distributions across time (i.e., sequences of the same age) also show a consistent signal of a stable population over time without an event near 1000 years BP (Fig. 4B). We also produced EBSPs for Native Americans that allow us to contrast the demographic history of the first Americans with that of their dogs. The EBSP of the human samples shows an increase 15,000 to 20,000 years BP with a slight decrease at ~500 years BP (Fig. 4C). The simulations of synthetic Native American HVR1 datasets show a similar population increase at ~15,000 years BP, but a much smaller or even absent decrease toward the most recent times (Fig. 4D).

**Discussion**

This study of the historical dynamics of Native American dogs has significantly increased the size and geographic diversity of genetic data from ancient dogs in the Americas. Of the nine haplotypes identified in the populations from JBG, APP, and DP, four are novel, which suggests that additional variation present in dogs in the Americas prior to European contact has yet to be identified. Additionally, our ancient dog sample from the Janey B. Goode site is the largest sample of dog mtDNA data from a single archaeological site. The fact that so many dogs were buried at this site suggests that the people who lived there placed considerable value on their canine companions. Critically, the large sample size also increases the likelihood that estimates of genetic diversity more accurately reflect the true genetic diversity of this dog population.

Notably, the increased sample size and expanded geographic distribution enabled us to identify putative founder haplotypes. Leonard et al. (2002) identified five putative founding haplotypes, two of which were present in ancient dogs and three of which were found in contemporary dogs, as well as a clade ‘a’ consisting of haplotypes of Latin American dogs unique to the Americas. These haplotypes were identified using a different nomenclature, such that some of these haplotypes have an identical hypervariable region to multiple dog haplotypes identified in Pang et al. (2009), which can make the founding haplotypes difficult to identify in some cases. According to guidelines established by Torroni et al. (1993a), haplotypes that fit these guidelines and are likely putative haplotypes are identified with an asterisk in Fig. 2D. Given that the most common haplotype is shared among dogs from the Midwest, Southeast and Southwest, as well as Siberia (the probable
source of the American dog populations; Forster et al., 1996; Leonard et al., 2002), this haplotype is likely a founding haplotype. Another haplotype, one shared with dogs from JBG, Alaska, and Peru, and identified by Leonard et al. (2002) as a putative founding lineage, is likely also a founder haplotype because it is widespread across the Americas. A third haplotype, also identified by Leonard et al. (2002), is shared between three Alaskan dogs and one dog from JBG, and could also be a putative founding lineage. Given that these founding haplotypes are characterized by only a portion of the mtDNA HVR, the haplotypes cannot be identified definitively unless complete mitogenomes of these dogs are sequenced. The clade ‘a’ identified by Leonard et al. (2002), however, seems to be a subhaplogroup localized to South America.

There are multiple outliers in the network that are distantly related to most of the other dog haplotypes from the early Americas. Some of them are closely related or identical to wolf haplotypes, as shown in Fig. 2A and B. These samples could indicate admixture with North American wolves or a separate domestication (or events) from North American wolves. This is most clearly demonstrated by the western Canadian haplotypes, which are almost identical to a North American wolf haplotype. Interestingly, the Mexican sample has an identical haplotype to a European wolf, but when this haplotype is the only one left different from a North American wolf haplotype. Given that the mtDNA only captures a fraction of an individual’s ancestry (i.e., maternal only) and only a portion of the hypervariable region of the mitochondrial genome was analyzed in our study, our results do not provide the resolution necessary to distinguish between the two possibilities of admixture and separate domestication in the Americas. Sequencing of other regions of the genome would be required to determine if these sequences derive from a single female wolf that interbred with domesticated dogs in the Americas, or if this haplotype represents the ancestor of domestic dogs in the Americas.

There are a total of eight outlier haplotypes shown in Fig. 2B that differ significantly from other ancient dog haplotypes, and there are multiple reasons why such outliers might be present in our dataset. First, these haplotypes represent dogs that came to the Americas via Siberia that exhibited haplotypes different from the other founding haplotypes, and represent additional founder lineages. Second, these dog haplotypes are shared with wolf haplotypes that have either gone extinct or have yet to be sampled and published in the literature. If they are shared with wolves, admixture or separate domestication are both viable possibilities. Interestingly, some of these haplotypes cluster more closely with dog haplotypes, suggesting that these dogs represent different founding haplotypes—they are likely too distantly related to the other haplotypes in the Americas to have diverged from another founding haplotype, but are more closely related to contemporary dog haplotypes than wolf haplotypes. Notably, some of the modern dog mtDNA haplotypes that are most closely related to the outliers in Fig. 3, such as A31, A121, and B28, are exclusively found in Asian dogs, further suggesting that these were founding haplotypes (Pang et al., 2009). However, it is surprising that wolf and dog haplotypes are so thoroughly intermixed in Fig. 3, when in Fig. 2 the wolf haplotypes seem far more distant from the dog haplotypes. The cases of admixture identified in the network explain some of this intermixing, but the similarities may also be due to the use of only the hypervariable region in this study. The relatedness between the dog and wolf haplotypes may be much lower than the tree indicates if the complete mitogenome were incorporated into the tree.

When comparing the two measures of genetic diversity (theta S and nucleotide diversity), the values are strongly positively correlated ($R^2 = 0.66, p = 0.0024$, see also values in Tables 4 and 5) across populations. Both values were calculated on a per nucleotide position scale. Interestingly, the correlation is stronger when excluding the sample from Tula, Mexico, which has a high theta S estimate relative to its estimated of nucleotide diversity. The dogs from Tula, Mexico, have mitochondrial sequences that differ greatly from one another in a very small sample size, which accounts for this difference and is unusual amongst the regional datasets.

Surprisingly, the genetic diversity measurements of the dog populations studied do not seem to follow any geographic pattern. For example, the Alaska, western Canada, Mexico, and Peru populations display high levels of diversity, whereas the sites in Bolivia, and the North American Southwest and Midwest have much lower levels of diversity. This may be due to sampling — some of the regions used for analysis had multiple archaeological sites, which might inflate the genetic diversity as compared with regions with one or few. However, as shown in Table 5, even when samples from only individual archaeological sites are used in the analysis, diversity levels remain high in Mexico and Alaska, while Illinois and coastal British Columbia have low levels of diversity. The measures of nucleotide diversity for JBG and DP are also lowered, reflecting the high frequencies of common haplotypes found at these sites, though diversity estimates remain low even when the haplotype frequency bias is removed because all of the haplotypes sampled from each site are closely related. This could indicate that the population from which the small number of closely-related female founders, or that variable breeding regimens of domesticated dogs were practiced very early across the Americas.

Examining the skeletal remains for morphological similarities or similar skeletal modifications could further support the possibility of selective breeding. Measurements of femora and humeri can be used as a reliable proxy for carnivore size (Von Valkenburgh, 1990). Femoral and humeral length in the JBG individuals have standard deviations of only 8 and 6 mm, respectively, indicating phenotypic homogeneity (data not shown). This homogeneity could be explained by inbreeding or selection, whether natural or artificial. Observations of village dogs that scavenge at human settlements but do not directly interact with humans suggest that they are all very similar in size, likely because a balance must be struck between being small enough to thrive on limited nutrients and being large enough to defend against other dogs (Coppinger and Coppinger, 2001). Humans could have also been directly selecting for a particular size of dog as well: given that JBG dogs were likely used for hauling supplies (Borgic and Galloy, 2004), perhaps the humans living there were selecting for dogs well-suited for hauling a given weight for long distances. An inbred population would be highly genetically similar and therefore phenotypically similar as well. Additionally, the dogs sampled from the DP site in this study come from a single shell midden, which represents only a small portion of the dogs recovered from the DP site that are available for study (Barta, 2006). These dogs could have been closely related and were therefore buried in the same shell midden, but the dog population at DP could have been much more genetically diverse overall. In future studies, comparing the phenotypic measurements and genetic data from other dog populations to genetic data will help determine the correlation between phenotypic and genetic variability in Native American dogs.

We performed Bayesian coalescent analysis to estimate the historical demography of dogs in the Americas using the methods implemented in BEAST. The EBSP shows a stable dog population across time, with a small dip around 1000 years BP. However, our subsequent analyses suggest that the genetic signal of the EBSP is biased by the sampling. Specifically, large numbers of dogs were sampled from the same time period with identical haplotypes (mostly at JBG), and this likely causes the ‘dip’ in the plot roughly 1000 years BP. We have two lines of evidence that support this conclusion. First, we analyzed simulated datasets consisting of random subsets of ancient samples (all modern samples were...
retained), which theoretically eliminated large numbers of duplicate sequences from the same ancient sampling period, which produced EBSPs with no discernible decrease in population size (SOM Fig 1B). Second, our analysis of synthetic datasets simulated under a constant population size and with the same sampling regime (i.e., with the same sampling dates) but without any correlation between sampling time and relatedness (i.e., samples from the same period were not more closely related than those from other sampling periods) also produced EBSPs without any consistent deviation from a constant population (Fig. 4B). Combined, these simulations suggest that the decrease in population size at ~1000 years BP is an artifact introduced by sampling bias (i.e., the correlation between sampling time and relatedness).

As posited above, there are many reasons why we should expect human and dog population dynamics to be correlated, as the histories of dogs and humans are intertwined. To investigate human history of the Americas at the same genetic resolution used in our analysis of dog history, we performed Bayesian coalescent analysis of the human HVR1 data used in O’Fallon and Fehren-Schmitz analysis of dog history, we performed Bayesian coalescent analysis of the human HVR1 dataset. As expected, the human EBSP shows an increase ~15,000 years BP, followed by a stable population size lasting nearly to the present day, with a late, non-significant dip in effective population size near the present. Interestingly, this EBSP differs from the EBSP produced from the complete human mitogenome, in which there is a clear population decline around the time of European contact (O’Fallon and Fehren-Schmitz, 2011). Our analysis of synthetic data simulated under a demographic model with a recent-post-Columbian population collapse of 50% also did not produce EBSPs that consistently reflect this event. Combined, these contrasting results suggest that the hypervariable region of mtDNA may not be expected to reveal fine-grained changes in recent population history, though it may be possible that larger samples of HVR1 sequences might contain enough signal to reliably reflect recent population histories.

When considering both the human and dog EBSPs, the population stability found in the dog plot is unexpected, as one would expect the dog population to increase in size over time, as the human population did. Intriguingly, this unexpected finding might indicate that humans were controlling dog matings and effectively breeding them, or that the population of dogs in the Americas quickly reached long-term carrying capacity. The stable population of dogs had a median effective population size of ~1000 female individuals at the time of the first dog burial in the Americas. This is consistent with other estimates of dog effective population size that suggest a global population of roughly 10,000 female dogs at the same time (Thalmann et al., 2013). Additionally, the coalescence date estimate for our dataset of American dogs is also surprising, given that by ~9000 years BP dogs should have been established across the Americas as they are thought to have arrived in the Americas ~15,000 years BP with humans. However, it is interesting that from 9000 years BP to the present, the population of dogs in the Americas roughly mirrors that of humans, in that both are stable for long periods of time.

Importantly, sequencing the hypervariable region alone captures a lot of diversity in a short stretch of sequence, but does not provide a full picture of mitogenome diversity. As demonstrated by SOM Table 1, multiple dog haplotypes have identical sequences in the region we studied, so it is possible that we are underestimating the diversity of dog mitogenomes present in the Americas, as was found true of human mitochondrial diversity as mitogenome sequencing became more routine (Samm et al., 2007; Achilli et al., 2008; Fagundes et al., 2008). This underestimation, combined with the possibility of breeding practices mentioned above, may mean that some of the putative founding haplotypes we have identified are not as frequent or as widespread as our results seem to indicate. Furthermore, if such population structure does exist, widespread sampling of dogs from multiple locations will be necessary to accurately characterize the diversity of dog founding mtDNA lineages. Calculating measures of population diversity using a short segment of DNA has revealed some interesting differences in diversity between populations, but it is pertinent now to sequence complete mitogenomes of these dogs to ensure that short nucleotide sequences have not biased these estimates. Ultimately, these analyses have confirmed that using complete mitogenomes can also provide a clearer picture of dog population history.

Given that dogs and humans have lived interdependently for thousands of years, dogs have potential for use as a proxy to test human models of migration. Comparison of human populations with the dog populations in this study could demonstrate similarities that show the utility of dogs as a complementary dataset for testing migration models. Notably, the JBG site also has human remains, and DNA analysis of these individuals is currently in progress. However, the human remains postdate the dog remains by at least 200 years at this site, and so the populations may not be directly comparable. In the cases of APP and DP as well, there are no human remains that have been found to be contemporaneous with the dog remains sampled for this study. However, this absence of human remains highlights the utility of using dogs as a proxy to learn more about how people lived in regions and time periods from which no human burials have been found.

Additionally, the haplotype distribution and diversity of dog haplotypes in a given region can allow us to infer human interactions with dogs, which can tell us more about the human population. Deliberate burial of dogs indicates that humans took care of the animals, and low levels of diversity can suggest deliberate breeding practices. If the dogs were being bred for a specific purpose, such as to haul sledges as in the case of the JBG dogs, it is possible that their owners were selecting for specific traits. If a single haplotype is frequent in multiple regions, it is possible that dogs migrated with humans to multiple locations. Haplotype distributions can also reveal instances of genetic drift, as seems to be the case in South America. Of the nine individuals currently sequenced from South America, eight of them are all part of the same derived clade. This founder effect has also been identified in human populations (Wang et al., 2007), supporting the idea that dogs and humans have traveled together and their populations have changed over time in similar ways.

Conclusion

Combining our data generated in this study with all published ancient dog haplotypes has revealed new diversity and multiple shared haplotypes across broad regions of the Americas. Some archaeological sites exhibit low levels of genetic diversity, suggesting the possibility of deliberate breeding practices in the area. The most frequently identified haplotype in the sample is likely a founding haplotype, especially since it is identical to that of an ancient Siberian dog, possibly from the same source population. The haplotypes have also been identified in human populations from Eurasia and possibly from the same source population. Two additional haplotypes that are not as frequent or as widespread, but still had a broad sampling, are also putative founding haplotypes. Additional outlier haplotypes that are infrequent but share similarities with modern dog haplotypes from Eurasia may also be possible putative founding haplotypes. This provides a minimum of three to five founding haplotypes. Given that each of these haplotypes is identical in sequence to multiple contemporary dog haplotypes, determining the nomenclature of the founding haplotypes should likely incorporate whole mitogenome sequences. Of the ~273 dog haplotypes that have currently been
identified, 29 can be found in dogs of pre-European contact Americas.

Multiple DNA sequences were identified that were identical to haplotypes of North American wolves, and could represent admixture with wolves or a separate domestication event(s). However, the hypervariable region of the mitochondrial genome lacks power to draw certain conclusions (i.e., poor resolution of fine haplotype structure) and as mitochondrial DNA represents only the direct maternal line of numerous ancestors, it is not possible to distinguish between admixture with wolves and a separate domestication event. Examination of other regions of the genome, such as autosomal SNPs or even the exome (the complete coding region of the genome) could better elucidate the evolutionary history of these dogs, as they are a result of multiple ancestral lineages.

The analysis of mitochondrial DNA haplotypes in this study provides insight to the population history of dogs in the Americas and brings us closer to a comparison between population histories of Native Americans and their dogs. However, the hypervariable region analyzed in this study is only a short segment of the mitochondrial genome. Direct maternal line of numerous ancestors, it is not possible to

distinguish between admixture with wolves and a separate domestication event. Examination of other regions of the genome, such as autosomal SNPs or even the exome (the complete coding region of the genome) could better elucidate the evolutionary history of these dogs, as they are a result of multiple ancestral lineages.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found online at doi:10.1016/j.jhevol.2014.10.012.

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