Genomic Data from Extinct North American *Camelops* Revise Camel Evolutionary History

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Abstract

Recent advances in paleogenomic technologies have enabled an increasingly detailed understanding of the evolutionary relationships of now-extinct mammalian taxa. However, a number of enigmatic Quaternary species have never been characterized with molecular data, often because available fossils are rare or are found in environments that are not optimal for DNA preservation. Here, we analyze paleogenomic data extracted from bones attributed to the late Pleistocene western camel, *Camelops* cf. *hesternus*, a species that was distributed across central and western North America until its extinction approximately 13,000 years ago. Despite a modal sequence length of only around 35 base pairs, we reconstructed high-coverage complete mitochondrial genomes and low-coverage partial nuclear genomes for each specimen. We find that *Camelops* is sister to African and Asian bactrian and dromedary camels, to the exclusion of South American camelids (llamas, guanacos, alpacas, and vicuñas). These results contradict previous morphology-based phylogenetic models for *Camelops*, which suggest instead a closer relationship between *Camelops* and the South American camelids. The molecular data imply a Late Miocene divergence of the *Camelops* clade from lineages that separately gave rise to the extant camels of Eurasia. Our results demonstrate the increasing capacity of modern paleogenomic methods to resolve evolutionary relationships among distantly related lineages.

Key words: **paleogenomics, *Camelops*, Pleistocene, arctic, phylogeny, morphology, camel evolution.**

Introduction

The western camel, *Camelops*, was the largest of the late Pleistocene North American camelids, until its extinction some 13,000 calendar years ago (13 ka), just before the end of the Pleistocene Epoch (Kooyman et al. 2012; Waters et al. 2015). Although its geographic range fluctuated, *Camelops* was widely distributed throughout western North America, from the subtropics of Honduras to the arctic latitudes of Canada (fig. 1, supplementary table S1, Supplementary Material online; Webb 1965; Webb and Perrigo 1984; Pinsof 1998; Graham and Lundelius 2010). Across this range, *Camelops* lived in a variety of habitats (Webb 1965; Zazula et al. 2011; Waters et al. 2015), where it fed on a mixed herbivorous diet of leaves, shrubs, and grasses (Zazula et al. 2011 and references therein). *Camelops* is one of the few megafaunal taxa that was demonstrably preyed upon by early humans in North America and has figured prominently in discussions of late Pleistocene megafaunal extinction (Kooyman et al. 2012; Waters et al. 2015).

Camelops was among the last surviving of the North American camels. The family Camelidae originated in North America during the Middle Eocene, approximately 46–42 Ma (Honey et al. 1998). Camelidae was taxonomically diverse, comprising as many as 13 now-extinct genera during the Miocene (Honey et al. 1998). Paleontological and mitochondrial DNA evidence together suggest that two of its constituent major tribes diverged in North America during the late Early Miocene, about 17.5–16 Ma (supplementary fig. S1, Supplementary Material online; Honey et al. 1998; Hassanin et al. 2012). Today, one tribe, the Camelini, is represented by the Afroasian dromedary (*Camelus dromedarius*) and wild and domestic bactrian camels (*C. ferus*, *C. bactrianus*, respectively), the presumed common ancestor of which first dispersed across the then-exposed Bering Isthmus into Eurasia during the Late Miocene, 7.5–6.5 Ma (Pickford et al. 1995; Rybczynski et al. 2013), The other major tribe, the South American Aucheniini, consists today of llamas (*Lama glama*), guanacos, (*L. guanicoe*), and vicuñas/alpacas (*Vicugna vicugna*) (Grubb 2005). The ancestor of these South American camelids probably dispersed into South America across the Isthmus of Panama during the Great American Biotic Interchange, perhaps just before the Pliocene/Pleistocene boundary around 2.7 Ma (Bell et al. 2004).

Satisfactory resolution of fossil camelid taxonomy and phylogeny has been impeded by rampant morphological

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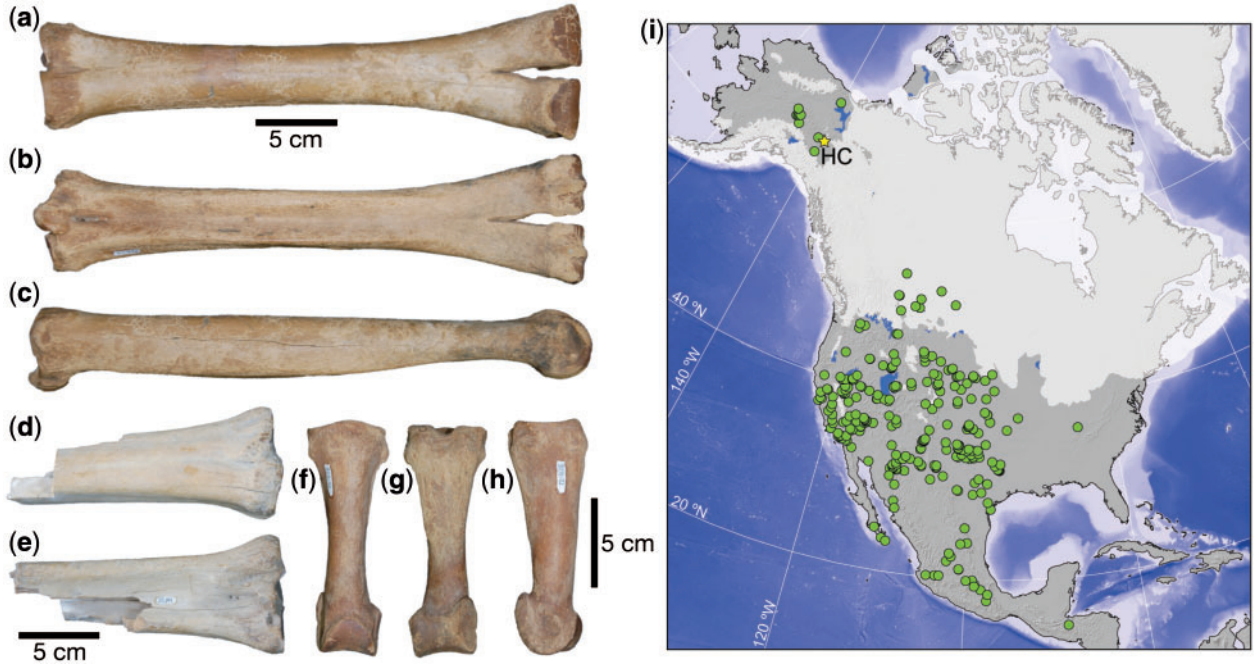

Fig. 1. The three Hunker Creek fossil specimens assigned to *Camelops* and the location of Hunker Creek in relation to other localities yielding *Camelops* specimens. YG 328.23: a complete, well-preserved left metatarsal (hindlimb cannon bone) in (*a*) anterior, (*b*) posterior, and (*c*) medial view. YG 29.199: a proximal fragment of a metacarpal (forelimb cannon bone) in (*d*) anterior, and (*e*) posterior view. YG 328.21: a complete and well-preserved proximal (first) phalanx or pastern bone in (*f*) anterior, (*g*) posterior, and (*h*) lateral view. (*i*) Known fossil localities of *Camelops* (circles), with late Pleistocene glacial limits and glacial lakes (white) from Ehlers et al. (2011). The Hunker Creek (HC) site is marked by a star. Locality data for *Camelops* are from the FAUNMAP database (Graham and Lundelius 2010), supplemented by additional sites from Alaska, Yukon, Mexico, and Honduras (supplementary table S1, Supplementary Material online).

parallelism, with virtually every "diagnostic" character being encountered in more than one proposed lineage (Harrison 1979; see supplementary information and supplementary fig. S1, Supplementary Material online, for an overview). Based on morphology, *Camelops* is widely recognized as a highly derived member of the Aucheniini, probably most closely related to Late Miocene *Alforjas* (~10 to 5 Ma; Honey et al. 1998; see also Harrison 1979; Breyer 1983; Voorhies and Corner 1986; Webb and Meachen 2004, but see Scherer 2013).

It has become increasingly routine to use molecular data to test morphology-based hypotheses concerning evolutionary relationships. In particular, ancient DNA (aDNA) and fossil proteins offer the possibility of testing phylogenetic hypotheses involving both living and extinct species (e.g., Orlando et al. 2003; Lister et al. 2005; Rohland et al. 2007; Campos et al. 2010; Llamas et al. 2015; Welker et al. 2015). Here, we evaluate three *Camelops* fossils, all lower limb bones recovered from a late Pleistocene, presumably Sangamonian Interglacial (~125 to 75 ka), context in subarctic Yukon, Canada. Fossils from interglacial deposits tend to be rare at higher latitudes, at least partly because the generally acidic nature of interglacial soils is not conducive to long-term preservation of bones or biomolecules (Lindahl 1993; Muhs et al. 2001). Consequently, there have been few successful attempts to recover aDNA from fossils of subarctic and arctic interglacial faunas (but see Rohland et al. 2007) despite this interval being well within the current temporal envelope for successful aDNA characterization (Orlando et al. 2013). Using a combination of morphological and paleogenomic analyses, we demonstrate that 1) these fossils can be assigned to *Camelops* with confidence on the basis of morphological and mensurational comparisons and that 2) *Camelops* is phylogenetically closer to *Camelus* (Camelini) than *Lama*/*Vicugna* (Aucheniini). Divergence dating indicates that the lineages of *Camelops* and *Camelus* separated during the Middle to Late Miocene.

Systematic Paleontology

Classification

Class: Mammalia; Order: Artiodactyla; Family: Camelidae; Subfamily: Camelinae; Genus: *Camelops* Leidy 1854.

Fossil Locality and Age

Three specimens of *Camelops* (YG 29.199, 328.21, and 328.23) were recovered from an active placer gold mine along Hunker Creek (64.019167 N, 139.158056 W), a waterway located near Dawson City, Yukon, Canada (fig. 1). Pleistocene vertebrate fossils are commonly uncovered at these localities by hydraulic stripping of frozen sediments, during mining operations designed to gain access underlying gold-bearing gravel (Froese et al. 2009). Mining in this manner does not proceed stratigraphically, with the result that there is usually only limited information for placing individual fossils in a precise chronological context. However, the fossil assemblage that yielded these *Camelops* fossils also includes steppe-bison (*Bison priscus*), considered to be chronologically restricted to the late

Table 1. Age Data, Endogenous DNA Percentage, and Genomic Coverage Statistics for the Three Hunker Creek *Camelops* Specimens.

Specimen Number	Radiocarbon Age (^{14}C Year BP)	Radiocarbon Lab Number	Reads		Mitochondria		Nuclear					
							Aligned to Wild Bactrian Camel			Aligned to Alpaca		
					% Reads Mapped	Coverage (×)[b]	Reads Mapped		Coverage (×)	Reads Mapped		Coverage (×)
			Raw	Filtered[a]			%	Per Mb		%	Per Mb	
YG 328.23	> 51,700	UCIAMS 117246	33,604,352	31,930,308	0.023	23.196	20.129	3,199.0	0.149	18.789	2,761.9	0.129
YG 29.199	> 49,900	UCIAMS 72416	24,773,604	23,529,508	0.030	21.177	13.185	1,544.0	0.066	12.262	1,328.2	0.057
YG 328.21	> 51,700	UCIAMS 117244	37,783,926	35,426,303	0.029	35.653	44.647	7,872.2	0.357	41.778	6,813.7	0.310
		Total	96,161,882	90,886,119								

NOTE.—The percentages of filtered reads that mapped (endogenous DNA) are reported prior to duplicate removal, whereas coverage statistics were calculated after duplicate removal. ^{14}C year BP, radiocarbon years before the present; Mb, megabase.

[a]Adapter dimers, reads with a quality score of <15 and length of <25 bp removed.

[b]As inferred from the iterative assembler.

Pleistocene (<130 ka; marine isotope stage 5/6 boundary; Lisiecki and Raymo 2005), as well as the less indicative woolly mammoth (*Mammuthus primigenius*), caribou (*Rangifer tarandus*), Dall sheep (*Ovis dalli*), and horse (*Equus* sp.). Radiocarbon dating of the camel specimens yielded nonfinite (> 50 ka) dates (table 1, but see supplementary information, Supplementary Material online). Although certainty is not possible, on paleoecological grounds (Zazula et al. 2014), we argue that the actual age of these fossils is likely to lie between roughly 130 and 75 ka, rather than to 50 ka.

Fossil Descriptions and Taxonomic Assessment

The three *Camelops* specimens (fig. 1*a*–*h*) all display diagnostic camelid morphology, and there can be no doubt about their familial placement. Comparison to homologous elements assigned to other fossil camelid taxa indicates that these specimens are most similar to *Camelops hesternus* (supplementary information and dataset S1, Supplementary Material online; Webb 1965; Breyer 1974; Voorhies and Corner 1986). In the absence of diagnostic cranial or dental material from this locality, however, we cannot place the Hunker Creek camel fossils unequivocally in a named species, although we provisionally designate them as *Camelops* cf. *hesternus*, following Harington (1997). The cautionary "cf." would be unnecessary if one were to adopt Pinsof's (1998) view that the several named species of *Camelops* are actually conspecific (as *C. hesternus*; see supplementary information, Supplementary Material online).

Results and Discussion

Characterization of *Camelops* aDNA

Genetic data recovered from the three Yukon *Camelops* specimens provided 21–35 × coverage of each mitochondrial genome and 0.07–0.36 × coverage of each nuclear genome. Endogenous DNA content ranged between 13% and 45% (table 1); molecules were very short (modal length of ~35 bp) and damaged, with between 30% and 34% of cytosines deaminated at the ends of reads (supplementary fig. S2, Supplementary Material online). The observed fragment length distributions and deamination patterns are consistent with those expected from aDNA (Dabney, Meyer, et al. 2013).

Phylogenetic Position of *Camelops* within Camelidae

Analysis of the full mitochondrial and low coverage nuclear genomes (figs. 2 and 3, Supplementary figs. S3 and S4 and tables S2 and S3, Supplementary Material online) of the three Yukon *Camelops* specimens establishes unequivocally that they are more closely related to African and Asian *Camelus* (Camelini) than to South American *Lama* and *Vicugna* (Aucheniini). This contrasts with the conventional interpretation of placing *Camelops* within the Aucheniini (e.g. (Harrison 1979, 1985; Voorhies and Corner 1986; Wheeler 1995; Honey et al. 1998; Scherer 2013) and suggests that a wider systematic revision of fossil camelids is required.

In the mitogenomic phylogeny, the *Camelops*–*Camelus* clade is recovered with strong statistical support regardless of analytical parameters (fig. 2 and supplementary figs. S3 and S4, Supplementary Material online), with 100% posterior probability and 98–100% maximum likelihood bootstrap support in the presence of an outgroup and 94% posterior probability support in analyses lacking an outgroup (supplementary figs. S3 and S4 and table S2, Supplementary Material online). We estimated nuclear phylogenies using pairwise transversion distances to minimize the influence of damaged sites in the low-coverage paleogenomic dataset. The branching order of the resulting phylogeny was concordant with that of the mitochondrial phylogeny, regardless of whether the wild bactrian camel or alpaca was used as a reference genome for mapping the *Camelops* reads (fig. 3; supplementary table S3, Supplementary Material online). More *Camelops* reads aligned to the wild bactrian camel genome than to the alpaca genome per megabase (table 1), again suggesting that *Camelops* and *Camelus* genomes are more closely related to each other than either is to *Lama*/*Vicugna*. We also note that the branches leading to *Camelops* were shorter when the *Camelops* reads were mapped to the alpaca genome than when mapped to the wild bactrian camel genome (fig. 3*b*). This is likely due to biased recovery of increasingly conserved regions of the genome as evolutionary distance increases between the reference genome and *Camelops* (Prufer et al. 2010).

The mitochondrial and nuclear genome analyses are in slight disagreement with respect to resolved relationships

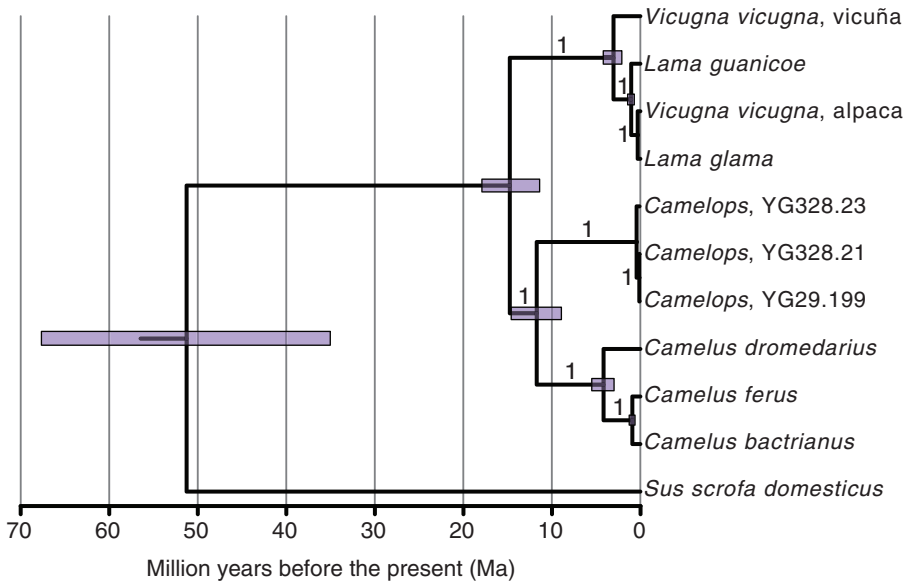

Fig. 2. Bayesian timetree of the extant Camelidae and extinct *Camelops* as inferred from whole mitogenomes. Here, a pig (*Sus scofra domesticus*) was included as outgroup and the analysis assumed a birth–death serially sampled speciation model and a median age of each *Camelops* specimen of 90,000 years. Bayesian posterior probabilities are shown along branches leading to clades for which monophyly was not enforced. Bars represent 95% highest posterior density credibility intervals for node heights.

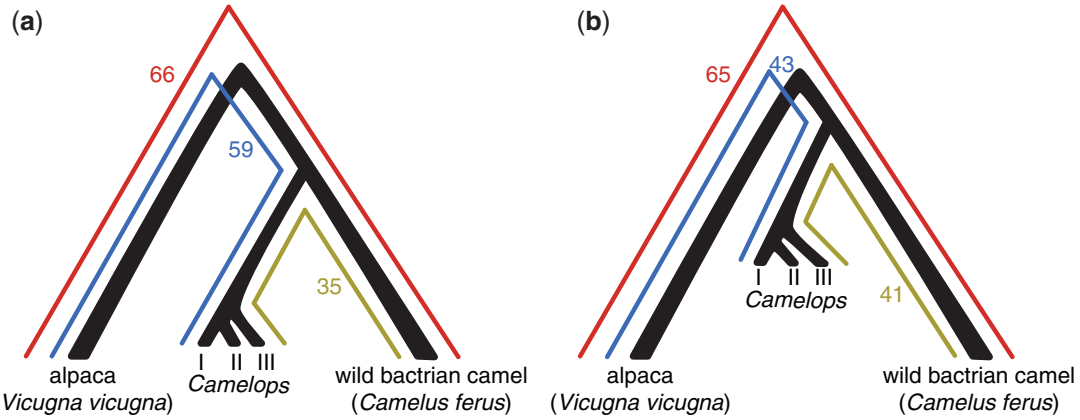

Fig. 3. Inferred phylogenetic relationships based on transversion pairwise differences between nuclear genomes of the three *Camelops* specimens, alpaca, and the wild bactrian camel, based on alignment to either the (*a*) wild bactrian camel or (*b*) alpaca reference genomes. The mean number of transversion differences per 10,000 sites are shown between wild bactrian camel-alpaca, *Camelops*-alpaca, and *Camelops*-wild bactrian camel (see also supplementary table S3, Supplementary Material online). *Camelops* specimens are I: YG 29.199, II: YG 328.23, and III: YG 328.21.

among the three *Camelops* specimens, with either YG 328.23 or YG 328.21 standing as sister to the remaining specimens, respectively (fig. 2 and supplementary figs. S3 and S4 and table S3, Supplementary Material online). This observation is consistent with a lack of lineage sorting, due to the three specimens probably having originated from the same population.

Dating the Divergence between *Camelops* and *Camelus*

Molecular clock-based analyses of the mitogenomic data from the Yukon fossils indicate that *Camelops* diverged from the lineage of Old World *Camelus* between 17.5 and 7 Ma (fig. 4; supplementary table S2, Supplementary Material online). This range spans the late Early Miocene through the middle Late Miocene (Gradstein et al. 2012). Crucially, analyses assuming a wide range of model parameters resulted in broadly congruent results, with overlapping 95% credibility intervals. We note that the analyses that assumed a birth–death (BD) serially sampled prior resulted in noticeably younger estimates than models assuming alternate speciation priors and that the choice of outgroup also had an observable effect on the divergence estimate when calibration set one was enforced (fig. 4; supplementary table S2, Supplementary Material online). Our divergence estimates between living camel clades (supplementary table S2, Supplementary Material online) are consistent with previous ages derived from analyses of whole genomes (Wu et al. 2014) and mitochondrial sequences (Ji et al. 2009; but see supplementary information, Supplementary Material online) from extant

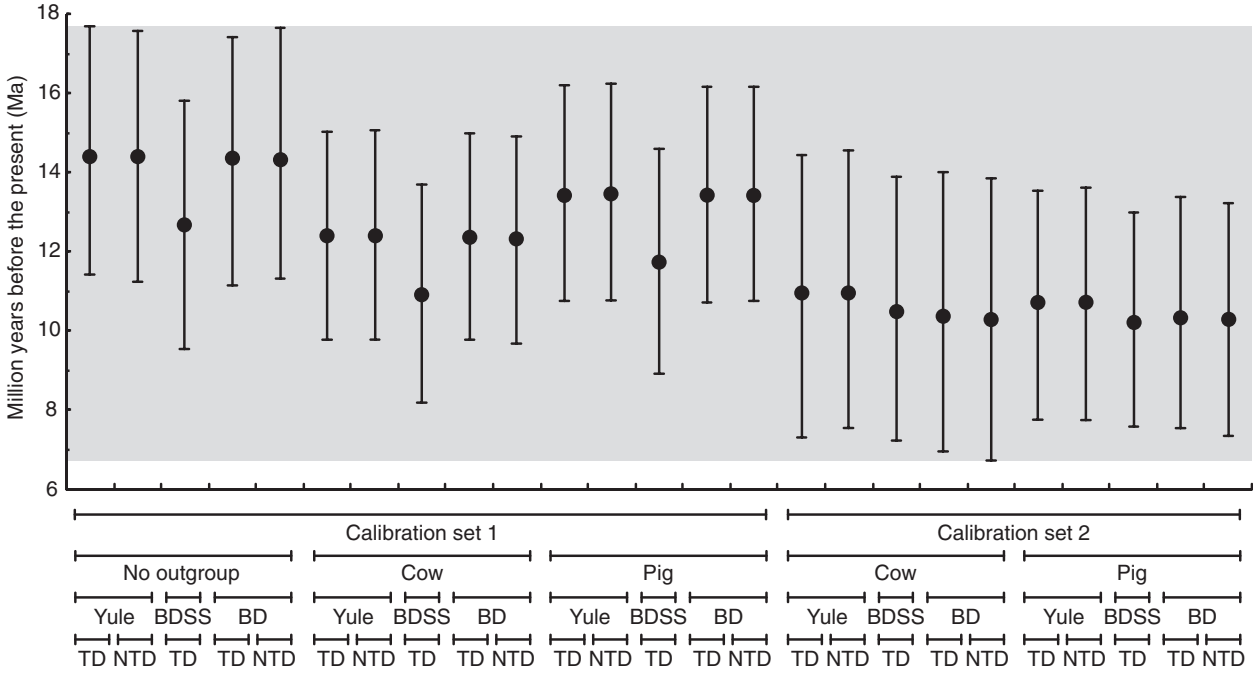

Fig. 4. *Camelops–Camelus* divergence estimates under various Bayesian model parameters (calibration set, outgroup, speciation model, and tip dating). Circles are mean height estimates and bars are 95% highest posterior density (HPD) credibility intervals. Gray background highlights the range of all 95% HPD credibility intervals (~17.5–7 Ma). BD, the birth–death speciation model; BDSS, the birth–death serially sampled speciation model; TD, tip dating enforced; NTD, tip dating not enforced.

species and are in broad agreement with the fossil record (Wheeler 1995).

To further test the *Camelops–Camelus* divergence estimate, we performed an additional analysis using the low coverage nuclear genome data. Assuming a genome-wide strict molecular clock, we inferred that these two lineages diverged between around 11 and 10 Ma (supplementary table S3, Supplementary Material online). This result is consistent with that derived from the mitogenomic data (fig. 4; supplementary table S2, Supplementary Material online).

Reconstructing the Evolutionary History of Camelidae

Both molecular and fossil record data suggest that crown group Camelidae arose in the late Early Miocene, around 17.5–16 Ma (supplementary fig. S1, Supplementary Material online; Honey et al. 1998; Hassanin et al. 2012; Wu et al. 2014). Our data support a subsequent divergence between the extant Old World camel (*Camelus*) lineage and the extinct *Camelops* clade prior to the late Late Miocene. This proposed divergence timing is consistent with hypotheses based on the fossil record that propose close affinities between Late Miocene *Paracamelus* and living *Camelus* (Titov 2008) and between Miocene *Alforjas* and *Camelops* (supplementary fig. S1, Supplementary Material online; Harrison 1979). *Paracamelus*, which is the presumed ancestor of living Old World camels, expanded from North America into Eurasia by approximately 7.5 to 6.5 Ma (Pickford et al. 1995). Eurasian *Paracamelus* later became separated from contemporaneous populations in arctic North America, probably as a result of the flooding of the Bering Isthmus approximately 5.5 Ma (Gladenkov et al. 2002). *Paracamelus* finally became extinct in the North American Arctic and Subarctic by the middle Pleistocene, roughly 1 Ma (Rybczynski et al. 2013). Stratigraphic and radiocarbon evidence suggest that *Camelops* and *Paracamelus* did not coexist in arctic and subarctic North America (Zazula et al. 2011; Rybczynski et al. 2013).

The fossil record suggests that *Camelops* first appeared during the middle Pliocene (~4.0 to 3.2 Ma) in southern North America (Thompson and White 2004) and achieved its maximum range during the late Pleistocene, with a reconstructed distribution from Alaska to Central America (fig. 1; supplementary table S1, Supplementary Material online). Fossils of *C. hesternus* in the northern part of its distribution in eastern Beringia (unglaciated Alaska and Yukon; fig. 1i) are very rare (Harington 1997), which may indicate either that populations were continuously present but always small or that *Camelops* dispersed into the subarctic only during brief interglacial intervals (Zazula et al. 2011). From a paleoecological standpoint, being able to distinguish between these alternatives is crucial, as the former implies a much greater capacity to withstand major climate change than does the latter.

Although *Camelops* was extinct throughout North America by roughly 13 ka (Waters et al. 2015), our nonfinite radiocarbon dates from Hunker Creek suggest that populations in eastern Beringia may have been locally extinct tens of millennia earlier, in parallel with the extinction chronology of American mastodon *Mammut americanum* (Zazula et al. 2014). This pattern may also apply to other rare fossil taxa found in eastern Beringia, such as Jefferson's ground sloth (*Megalonyx jeffersonii*) and the giant beaver (*Castoroides*

ohioensis; Harington 1990). However, additional dated remains from these species will be necessary to test this hypothesis. A better understanding of the timing of local versus regional extinctions has implications for interpreting continuity and change in the North American biota, including understanding the timing and significance of anthropogenic factors in forcing megafaunal extinctions.

Materials and Methods

This section provides an overview of the methods of this study; full details can be found in the supplementary information, Supplementary Material online.

Radiocarbon Dating

To generate radiocarbon dates for the fossil *Camelops* specimens, we extracted approximately 150 mg of collagen from each bone using a combination of standard Longin (1971) methods and ultrafiltration (Beaumont et al. 2010). The prepared collagen was radiocarbon dated at the KECK Accelerator Mass Spectrometry (AMS) Laboratory (University of California, UC, Irvine).

DNA Extraction, Library Preparation, and Sequencing

We followed standard protocols for aDNA research, outlined in Cooper and Poinar (2000). In a purpose-built aDNA facility, we extracted DNA from 100 to 120 mg of bone powder following Dabney, Knapp, et al. (2013) and constructed DNA libraries following Meyer and Kircher (2010). We sequenced the libraries using the Illumina HiSeq-2500 platform at UCSF.

Mitogenomic Reconstruction and Phylogenetic Analysis

We combined the sequenced reads from all samples and sequencing runs and used these to reconstruct the *Camelops* mitochondrial genome. We merged, adapter trimmed, and mapped each read to four extant camelid reference mitogenomes using an iterative assembler (Green et al. 2008). We then combined the resulting alignments to produce a draft of the *Camelops* mitogenome. Independent remapping of reads from each sample to the draft mitogenome, as well as visual inspection, gene annotation, and assessment of DNA fragment length distributions and deamination patterns (supplementary fig. S2, Supplementary Material online), were all consistent with what is expected from authentic aDNA sequences.

We aligned the three Yukon *Camelops* mitogenomes to seven extant camelid and ten other artiodactyl mitochondrial genomes collected from GenBank (supplementary table S4, Supplementary Material online). We partitioned the alignments into four partitions: control region, coding regions, rRNAs, and tRNAs. We then used PartitionFinder (Lanfear et al. 2012) and jModelTest (Darriba et al. 2012) to identify the most suitable model of molecular evolution for each partition (supplementary table S5, Supplementary Material online). Assuming these models, we then ran Bayesian and maximum likelihood phylogenetic analyses in MrBayes (Ronquist et al. 2012) and RAxML (Stamatakis 2014), respectively, to test for consistent camelid topologies under different combinations of three conditions: 1) including or excluding an outgroup taxon; 2) including or excluding the control region partition; and 3) using both a rate heterogeneity parameter (gamma) and a proportion of invariant sites or using only the rate heterogeneity parameter.

Phylogenetic Inference from Nuclear Genomes

To assess the phylogenetic relationships among Yukon *Camelops* and extant species in the Camelini and Aucheniini based on low coverage nuclear genomes, we calculated mean pairwise transversion differences between all pairs of *Camelops* specimens and the wild bactrian camel (Camelini) and alpaca (Aucheniini) reference genomes. To test for mapping bias that might lead to underestimation of the divergence between *Camelops* and the reference genome, we mapped all *Camelops* shotgun reads to both the wild bactrian camel and alpaca reference genomes. To calculate the divergence between the reference genome sequences, we created artificial datasets in which short fragments mimicking Illumina sequencing reads were sampled from each of the two reference genomes. These artificial "reads" were then remapped to the alternate reference genome. In inferring pairwise distances between genomes, we restricted our analyses to transversions so as to prevent cytosine deamination from biasing analyses (Dabney, Meyer, et al. 2013).

Divergence Dating

We performed Bayesian estimation of the divergence time between *Camelops* and *Camelus* in BEAST (Drummond et al. 2012) using alignments of whole mitogenomes partitioned as above. Analyses were restricted to the camelids, except for variations that included a single outgroup. We ran BEAST analyses assuming different combinations of parameters: 1) calibration set, 2) the presence/absence of an outgroup, 3) assuming a yule, BD, or BD serially sampled speciation model; and 4) using an estimated age of each *Camelops* specimen (90 ± 40 ka) as prior information or not. We used two calibration sets. The first set assumed a divergence between the Camelini and Aucheniini of 17.5 Ma, using a normal prior with a standard deviation of 1.52 Ma, so as to sample between 15 and 20 Ma with 90% probability, following Honey et al. (1998). The second set assumed a camelid-outgroup divergence of 59 Ma, using a lognormal prior with a log standard deviation of 0.07, so as to sample between 52.5 and 66 Ma with 90% probability, following Benton et al. (2015). The second calibration set also assumed a dromedary-bactrian camel divergence of 4.4 Ma, using a normal prior with a standard deviation of 1.43 Ma, so as to sample between 1.6 and 7.2 Ma with 95% probability, following Wu et al. (2014).

Finally, we further estimated the timing of divergence between *Camelops* and *Camelus* using the low coverage nuclear genome data aligned to the wild bactrian camel genome and assuming a genome-wide strict molecular clock. The divergence was calculated for each specimen by multiplying the Camelini–Aucheniini split (17.5 Ma; Honey et al. 1998) by

the ratio of transversions between wild bactrian camel-*Camelops* and alpaca-*Camelops* (supplementary table S3, Supplementary Material online).

Supplementary Material

Supplementary information, figures S1–S4, tables S1–S5, and dataset S1 are available at *Molecular Biology and Evolution* online (http://www.mbe.oxfordjournals.org/).

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