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Cite this article: Wellman HP, Austin RM, Dagtas ND, Moss ML, Rick TC, Hofman CA. 2020 Archaeological mitogenomes illuminate the historical ecology of sea otters (*Enhydra lutris*) and the viability of reintroduction. *Proc. R. Soc. B* **287**: 20202343. https://doi.org/10.1098/rspb.2020.2343

Received: 20 September 2020
Accepted: 9 November 2020

Subject Category:
Genetics and genomics

Subject Areas:
genetics, ecology

Keywords:
ancient DNA, mitochondrial genomes, marine mammals, applied zooarchaeology, conservation, translocation

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Electronic supplementary material is available online at https://doi.org/10.6084/m9.figshare.c.5221702.

THE ROYAL SOCIETY PUBLISHING

Archaeological mitogenomes illuminate the historical ecology of sea otters (*Enhydra lutris*) and the viability of reintroduction

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Genetic analyses are an important contribution to wildlife reintroductions, particularly in the modern context of extirpations and ecological destruction. To address the complex historical ecology of the sea otter (*Enhydra lutris*) and its failed 1970s reintroduction to coastal Oregon, we compared mitochondrial genomes of pre-extirpation Oregon sea otters to extant and historical populations across the range. We sequenced, to our knowledge, the first complete ancient mitogenomes from archaeological Oregon sea otter dentine and historical sea otter dental calculus. Archaeological Oregon sea otters ($n = 20$) represent 10 haplotypes, which cluster with haplotypes from Alaska, Washington and British Columbia, and exhibit a clear division from California haplotypes. Our results suggest that extant northern populations are appropriate for future reintroduction efforts. This project demonstrates the feasibility of mitogenome capture and sequencing from non-human dental calculus and the diverse applications of ancient DNA analyses to pressing ecological and conservation topics and the management of at-risk/extirpated species.

1. Background

(a) Reintroductions and applied archaeology

The extinction and extirpation of animals and plants, and associated ecological degradation, are increasing at a rapid rate [1]. Responses to these challenges include reintroductions, translocations and other strategies used to bolster or re-establish populations of threatened or endangered species [2]. Significant challenges exist related to animal homing instincts, source population choice, predation and reproductive failures [3–6].

Genetic analyses are valuable for assessing reintroduction and translocation viability, and for documenting the impact of genetic rescue [6–8]. For example, microsatellites in desert tortoises have been used to show poor reproductive success in translocated males [5], while genomic approaches, including RADSeq and transcriptomics, highlight the importance of local adaptation in other organisms [9]. To avoid outbreeding depression and translocation failure and to account for potential local adaptation, genetic studies suggest that reintroduction efforts should maximize ecological similarity and minimize population divergence times between source and sink populations [7,8,10]. Past extirpation events may present particular challenges owing to the lack of recent genetic data on the extirpated population. Ancient DNA approaches provide a powerful method to bridge temporal gaps and provide relevant data, such as identifying

appropriate source populations [11] and documenting genetic diversity before extirpation [12].

We present a novel approach integrating new methods and sources of ancient DNA to inform reintroductions of the sea otter (*Enhydra lutris*). The sea otter, a keystone species in kelp forest ecosystems, was hunted to the verge of extinction around the Pacific Coast during the maritime fur trade but has yet to re-occupy a large portion of its former range [13]. Our study demonstrates the importance of an applied analytical toolkit for investigating twenty-first century global extirpations and efforts to repair ecological degradation and disruption.

(b) The sea otter on the Pacific Coast

Prior to the nineteenth century, the sea otter occurred along the coast from Japan to northern Mexico [14]. Intensive hunting by Russian and Euro-American companies during the maritime fur trade (1741–1911) severely depleted sea otter populations, resulting in genetic bottlenecks [12,15–18], and triggering profound ecological and socio-cultural changes. Sea otters are an ecological priority owing to their role as a keystone species in the kelp forest ecosystem: sea otters eat urchins (benthic echinoderms) which graze on kelps, thus filling an important role in near shore community structuring [14,19]. Kelp forests protect coastlines from erosion [19], support biodiversity, and provide carbon fixation [20]. Disruptions to kelp forest ecosystems, whether through the loss of sea otters or other factors (e.g. climate change), are cause for great concern. Socio-cultural consequences of the fur trade included disruption and dismantling of Indigenous social-ecological and economic systems at the hands of colonial powers [21], and in some contexts, Indigenous peoples were coerced into hunting on behalf of fur companies [22,23]. Reviving sea otter populations to revitalize coastal ecosystems remains a conservation priority in the present, and a recent study suggests that reintroducing sea otters yields a net ecological/economic gain [20].

By the mid-twentieth century, sea otters were patchily distributed throughout their original range owing to rebound and conservation measures. Northern sea otter sub-populations (*E. l. kenyoni*) in the Aleutian Islands and southern sub-populations (*E. l. nereis*) in parts of California survived peak hunting in the late nineteenth century because of their geographical isolation [24]. In the 1960s–1970s, biologists reintroduced sea otters from southcentral and southwest Alaska to southeast Alaska, British Columbia (BC) and Washington, and successfully re-established populations in parts of their former range. However, two 1970s reintroduction attempts at Port Orford and Cape Arago in southern Oregon failed [24,25]. The 'most plausible explanation' for the Oregon failure was emigration (the sea otters' attempt to return to their original range/habitat) and small post-release populations that subsequently collapsed [25]. The translocated northern sea otters may also have lacked adaptations suitable for their new Oregon coast habitat [25]. Today, sea otters remain extirpated in Oregon. The species is listed as endangered on the International Union for Conservation of Nature's (IUCN) Red List [26], and the Oregon Endangered Species Act (ORS 496.171–496.192) lists the Oregon sea otter as 'threatened'. There is growing interest in assessing whether reintroducing sea otters to Oregon is desirable and/or feasible. The Elakha Alliance non-profit is conducting a feasibility study as a step towards reintroduction, coastal ecological restoration and cultural revitalization with the partnership and support of the Confederated Tribes of Siletz and Coquille Indian Tribe [27,28].

Sea otter use and significance to tribal groups in Oregon are documented in archaeological and historical records, and tribal stories and oral histories [29–33]. Alaska Native and First Nations groups also have vested interests in sea otter conservation, use and management [21,34–36]. However, both Native and non-Native stakeholders are concerned about sea otter predation on commercially fished invertebrates [20,37,38], such as Dungeness crab in Alaska [35]. Because sea otters in Oregon were extirpated by the end of the fur trade (*ca* 1876, but possibly as late as 1906 [14]), there are some gaps in cultural and ecological knowledge pertaining to the species; the evidence is limited and few fur-trade era specimens or records are available for the study. As a result, available historical and archaeological Oregon sea otters represent a valuable, but often overlooked, source of data [30].

Reintroductions and subsequent management are complicated efforts involving many factors and stakeholders. Our study seeks to address a key aspect of Oregon reintroduction discussions: which post-fur trade sea otter populations are most closely related to the original (pre-fur trade) Oregon sea otter population and should serve as a source for reintroductions? We present a temporal perspective and dataset by recovering complete mitogenomes from archaeological (Late Holocene) and nineteenth-century fur trade Oregon sea otters and compare them to post-fur trade (twentieth-century and modern) sea otters to determine the relationships between populations.

(c) Previous studies

Mitochondrial DNA (mtDNA) and microsatellite analyses demonstrate that sea otter populations vary genetically along the northwest Coast [12,16,18,39–41]. Larson *et al*. [41] identified four modern sea otter mtDNA haplotypes on the northwest Coast. Valentine *et al*. [39] analysed a 222 base pair (bp) region of mtDNA from 16 archaeological Oregon sea otters and found four haplotypes: a California genotype represented by 11 Oregon individuals, an Alaska genotype represented by two Oregon individuals, and two new genotypes represented by two and one Oregon individuals, respectively. Valentine *et al*. [39] concluded the archaeological Oregon sea otters were, therefore, more closely related to the California sea otters, and future reintroductions using California sea otters might be more successful. Larson *et al*. [16] performed microsatellite analyses on pre-fur trade and modern sea otters, and found that gene flow occurred between Oregon, California and Alaska sea otters, including between Oregon and northern populations. Beichman *et al*. [15] identified specific aquatic adaptations and low genomic diversity in modern populations. Morphometric studies of sea otters have demonstrated some phenotypic traits vary along a latitudinal cline on the Pacific Coast, with Oregon sea otters intermediate by varying degrees [42–44]. We build on this research, presenting, to our knowledge, the first complete ancient mitogenomes for Oregon sea otters and demonstrate a new minimally destructive sampling technique using dental calculus.

(d) Current study

To expand upon and contribute to the current understanding of pre-fur trade Oregon sea otters, we sequenced complete

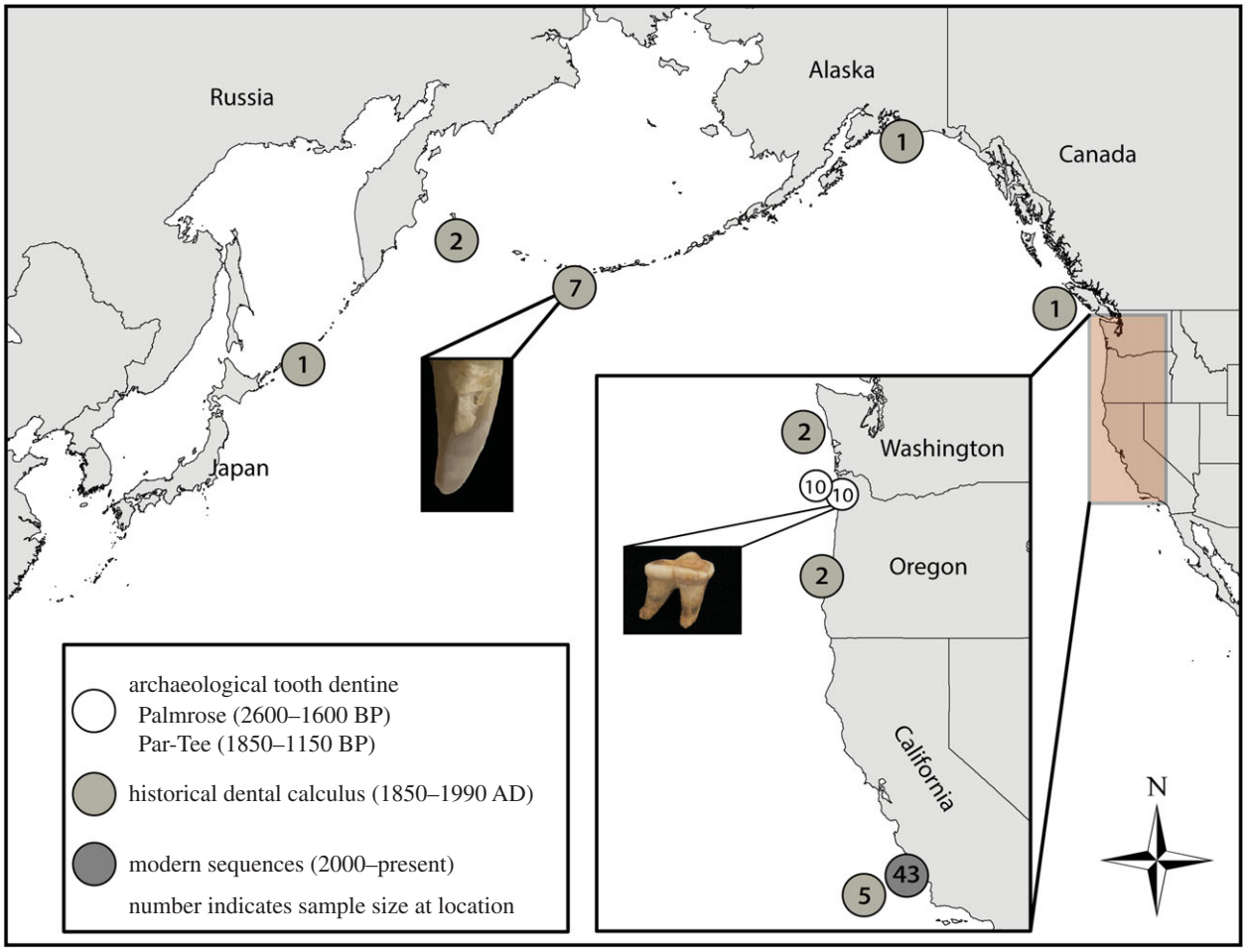

Figure 1. Map showing geographical origins of sea otter archaeological dentine (2600–1600 BP; 1850–1150 BP) and historical dental calculus (1850–1990 AD), as well as published modern (2000–present) sequences. Made in ArcMap 10.0/Adobe Illustrator; data from Natural Earth and U.S. Census Bureau. (Online version in colour.)

mitogenomes from 20 archaeological sea otter teeth (tooth dentine). We sampled 10 right lower first molars (M_1s) from the Par-Tee site (35CLT20) (1850–1150 cal BP; [45]) and 10 right M_1s from the Palmrose site (35CLT47) (2600–1600 cal BP; [46,47]). Par-Tee and Palmrose are located adjacent to each other in northern Oregon (figure 1). These sites were excavated in the 1960s through to the 1970s [48] and the faunal remains are abundant and well-preserved [43,49–51]. We compared the archaeological Oregon mitogenomes to 21 historical Pacific Coast mitogenomes captured and sequenced from dental calculus for this study (table 1; figure 1) and previously published modern California mitogenomes [18]. These historical mitogenomes include sea otters from the end of the fur trade (just prior to extirpation) and the post-fur trade era, including several 1960s Amchitka Island sea otters [14,52]. Amchitka sea otters were reintroduced to southeast Alaska, BC, and Washington, and therefore probably reflect present genetic diversity in those areas [16]. Historical pre-extirpation Oregon sea otter specimens are a rare and unique data source, and dental calculus was used to minimize destructive sampling to these specimens. Sea otter mitogenomes are assumed to accurately reflect geographical origin as they are maternally inherited, and while male sea otters may travel upwards of 100 km [53,54], females tend to maintain small home ranges and geographical fidelity (extending approximately 20 km along the coast and approximately 0.3 km offshore) [55–57]. Based on previous findings [16,39,42–44], we hypothesized that the archaeological Oregon sea otters would share mitochondrial lineages with both California and northern Pacific Coast populations, but more with the latter. We also anticipated that the archaeological specimens would exhibit greater genetic diversity compared to the available modern California specimens [18] owing to past bottlenecks [15,16,58].

2. Methods

Archaeological tooth dentine was sampled at the Laboratories of Molecular Anthropology and Microbiome Research (LMAMR) at the University of Oklahoma, Norman, in the dedicated sample preparation area following standard ancient DNA contamination protocols [59,60]. Dental calculus was sampled on location in museum research collections following a calculus-specific sampling protocol designed to reduce contamination (description in the electronic supplementary material with photos/specimen metadata). Dental calculus contains both endogenous (host) and microbial DNA [61–63] and can be removed without destruction to the specimen, preserving the integrity of rare museum collections while also addressing research questions regarding biodiversity/conservation biology. Owing to differential preservation of endogenous DNA in dental calculus [63] and documented degradation of DNA in calculus museum specimens [64], the historical DNA was extracted and sequenced following ancient DNA protocols and workflows.

Ancient and historical DNA extraction and library construction were performed in the LMAMR Ancient DNA Laboratory, a dedicated, six-room ISO-6 class cleanroom custom-built for

Table 1. Historical museum specimens from the National Museum of Natural History (NMNH) and the Santa Barbara Museum of Natural History (SBMNH).

museum	acc no.	sex	date collected	location	haplotype
NMNH	188636	F	—	Kurile Islands, Japan	22
NMNH	A49492	—	—	Copper Island, Bering Sea (Russia)	—
NMNH	206458	—	1911	Commander Islands (Kamchatka Peninsula)	17
NMNH	285441	F	7 Apr 1949	Amchitka Island, AK	—
NMNH	285469	M	26 Apr 1949	Amchitka Island, AK	3
NMNH	285470	—	26 Apr 1949	Amchitka Island, AK	5
NMNH	396641	M	28 Sep 1977	Green Island, Prince William Sound, AK	6
NMNH	527126	F	17 Jun 1960	Nagai Island, Eagle Harbor, AK	—
NMNH	527134	F	22 Jan 1962	Amchitka Island, AK	1
NMNH	527162	F	6 Feb 1962	Amchitka Island, AK	2
NMNH	527170	F	10 Feb 1962	Amchitka Island, AK	4
NMNH	256971	—	*ca* 1889[a]	Vancouver Island, BC	21
NMNH	93954	M	4 Jun 1898	Point Granville, WA	18
NMNH	188634	—	1897	Strait of Juan de Fuca, WA	—
NMNH	A3643	—	*ca* 1859[b]	Port Orford, OR	20
NMNH	A13460	—	*ca* 1874[b]	Oregon	19
SBMNH	1922F	F	1978	Cooper Point, CA	27
SBMNH	1366F	F	—	—	—
SBMNH	1367M	M	1977	Oso Flaco Creek, CA	26
SBMNH	3053	M	1983	Sunset State Beach, CA	25
SBMNH	3057M	M	1983	Point Piedras Blancas, CA	23

[a]Collected by T. T. Minor/Dr Thomas Minor. Dr Thomas Taylor Minor was a prominent Seattle physician and the presumed collector. We assigned his year of death (1889) as an approximate date.
[b]Based on additional information found in *Mammals and Life Zones of Oregon* by Vernon Bailey [29].

ancient DNA and microbiome research. Detailed procedures are provided in the electronic supplementary material. In brief, DNA was extracted from dental calculus and dentine using a protocol described in Morales *et al*. [65]. DNA extracts were converted into dual indexed Illumina sequencing libraries and captured using a custom in-solution biotinylated RNA bait set (Arbor Biosciences). Captured libraries were sequenced on an Illumina MiSeq with 2 × 150 bp chemistry.

The raw fastq files were quality filtered using the program AdapterRemoval2 (v. 2.1.7) [66] and mapped using bwa (v. 0.7.17) [67] with ancient DNA parameters to the published modern sea otter mitogenome [68] (electronic supplementary material, table S2). DNA authenticity was assessed using the program MapDamage2 [69] and fragment length plots (electronic supplementary material, figures S4 and S5). Consensus sequences were called from rescaled bam files in Geneious (v. 11.1.4) and aligned with MAFFT (v. 7.308) [70,71]. This alignment was stripped for identical sites and ambiguities and rendered into a network (figure 2) using the median-joining algorithm in PopArt [72,73]. Haplotype diversity was calculated in DnaSP (v. 6) [74]. In order to explore and visualize the temporal signal associated with haplotype diversity, we used TempNet in R (v. 3.6.3) (figure 3). We attempted estimating divergence times with BEAST (v. 1.10), but this dataset violated clocklike assumptions as tested with TempEst (v. 1.5.3). Raw sequence data are available through the NCBI Short Read Archive (SRA) under BioProject accession PRJNA550086. Consensus sequences and the alignment used for analysis (ModAlign.fa) are available from the Dryad Digital Repository (https://doi.org/10.5061/dryad.djh9w0vxz [75]).

3. Results

Eighteen of the 20 archaeological specimens and 16 of the 21 historical specimens yielded complete mitogenomes suitable for analysis. The median-joining network analysis (figure 2) illustrates the relationships between the sea otter mitogenomes generated for this study, previously published California mitogenomes [18], and the reference mitogenome [68]. The network analysis yielded 27 haplotypes: 10 (haplotypes 7–16) represent archaeological Oregon individuals, five (haplotypes 23–27) represent historical/modern California, six (haplotypes 1–6) represent historical Alaska, two (haplotypes 19 and 20) represent historical Oregon, and haplotypes 18, 17, 21 and 22 each represent historical Washington, Russia, BC and Japan, respectively (electronic supplementary material, table S3). Of the 10 archaeological Oregon haplotypes, six are unique to single individuals (7,9,11,12,15,16), two represent five and three individuals, respectively (9 and 14), and the final two haplotypes (10 and 13) include two individuals each. The mitogenomes of the historical samples occur as expected in the network given their geographical origins, except for the historical individuals from Russia and Japan. The archaeological Oregon/northern haplotypes show substantial separation from California haplotypes. The network also demonstrates high genetic diversity in the archaeological Oregon samples.

The TempNet analysis (figure 3) shows two shared haplotypes through time. The first (haplotype 9) includes three sea

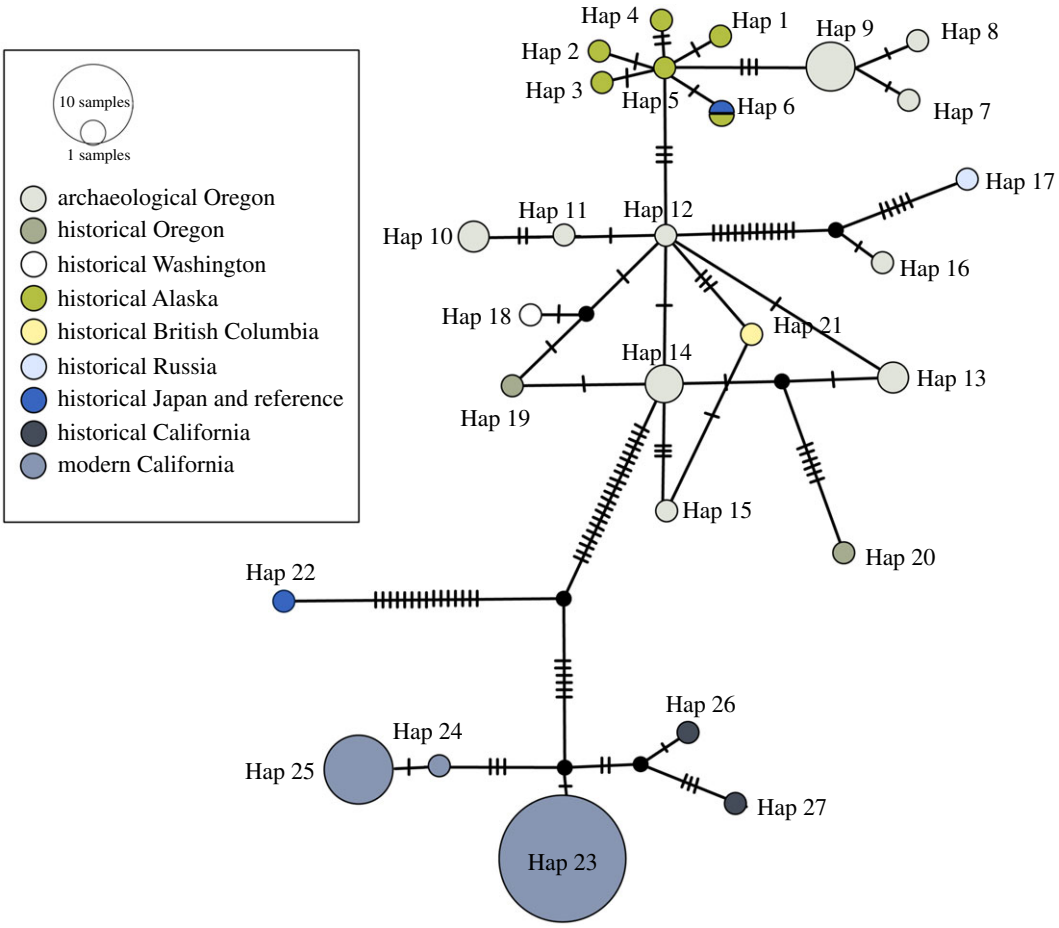

Figure 2. Median-joining network of archaeological Oregon, historical and modern haplotypes. Node size represents haplotype frequency corresponding to table 1 and electronic supplementary material, table S3. Hash marks represent nucleotide changes between haplotypes. The reference mitogenome (Yonezawa *et al*. [68]) came from a sea otter in the Toba Aquarium, Mie, Japan, but shares haplotype 6 with an Alaskan sea otter. (Online version in colour.)

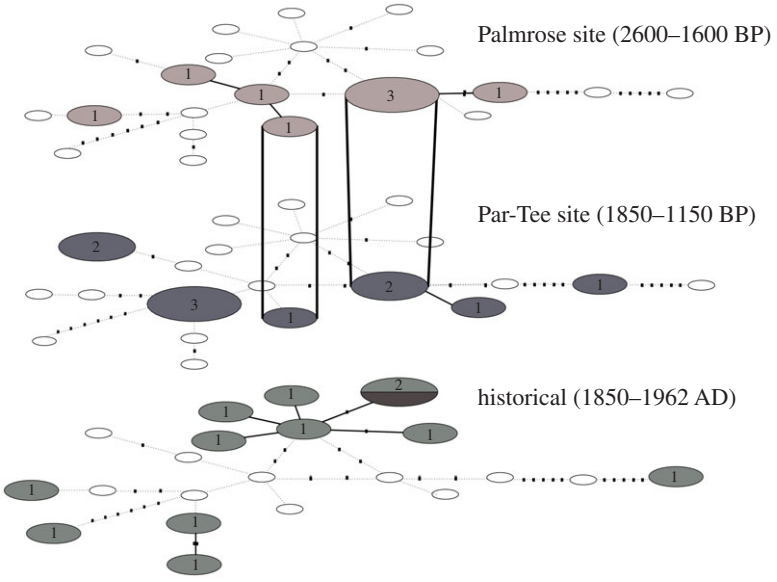

Figure 3. TempNet analysis showing shared haplotypes between archaeological (2600–1600 BP and 1850–1150 BP, respectively) and historical sea otters (1850–1962 AD). Circles represent the haplotypes in the median-joining network; colours indicate time periods of haplotypes. Haplotype overlap (bold lines) occurs between two Palmrose and Par-Tee haplotypes (haplotypes 9 and 13 in figure 2). California samples were omitted due to divergence from archaeological and historical northwest coast haplotypes.

otters from Palmrose and two from Par-Tee, and the second (haplotype 13) includes one individual from each site. There are no shared haplotypes between historical and archaeological individuals, despite the proximity of Oregon and northern haplotypes in the network (figure 2). The archaeological Oregon sea otters contain more overall haplotype diversity (Hd = 0.91) compared to the modern California samples (Hd = 0.44).

4. Discussion

(a) Mitogenome haplotype distributions

The mitogenome results provide new insights into archaeological/pre-extirpation Oregon sea otters. As hypothesized, the Oregon sea otter haplotypes are distinct from California haplotypes, and form several clusters with northern haplotypes in the network analysis.

Archaeological Oregon haplotypes 7, 8 and 9 (representing a total of seven individuals from both sites) are more closely related to the Alaska haplotypes (specifically haplotype 5, a 1949 Amchitka Island sea otter). Archaeological haplotypes 10–15 cluster with the historical Washington and BC haplotypes (18 and 21) and historical Oregon haplotype 19, all of which date close to extirpation (*ca* 1874–1898); this clustering is unsurprising given documented gene flow between northern populations prior to fur trade bottlenecks [16]. Historical Oregon haplotype 20 (*ca* 1859) is comparatively distant from this historical/archaeological cluster but was collected from Port Orford in southern Oregon, possibly reflecting variation on a latitudinal cline. Archaeological Oregon haplotype 16 is also distinct: it is closely associated with Russia haplotype 17 (collected 1911), and prior studies have indicated gene flow between archaeological Oregon and Russia populations occurred [16]. Interestingly, Japan (haplotype 22, no date) is separated from all other haplotypes including Russia, while the reference genome from a sea otter from the Toba Aquarium in Japan shares haplotype 6 with a 1977 historical Alaska sea otter. Overall, the distribution of haplotypes within the network analysis indicates close associations between the archaeological Oregon sea otters and pre-extirpation sea otters from northern populations, especially those immediately north of Oregon (Washington and BC), as well as the post-fur trade historical Alaska sea otters used for reintroductions.

(b) Pre-contact sea otter acquisition

Previous studies have documented Oregon sea otters sharing traits and experiencing gene flow with northern populations [16,44]. This gene flow along the coast may be responsible for the similar genetic signatures between groups, but animal and/or human behaviour may also be a factor. It is feasible sea otters travelled from southeast Alaska and were hunted in Oregon [53] (yielding the Alaska/Oregon cluster), but it seems unlikely a sea otter from populations further northwest would do so, especially in large numbers. Ethnographic data suggest that Oregon tribes (especially those in the Columbia River trading area) were the source, rather than recipients, of traded pelts [32,76], but it is possible pre-contact groups on the Pacific Rim/northwest coast may have moved animals (or in this case, their teeth or other parts) across long distances through trade networks. For example, the famous whale saddle wood carving from Ozette, WA, is inlaid with more than 700 sea otter teeth, mostly molars [77]. Such teeth could have been traded widely, perhaps as a symbol of the wealth/status associated with sea otter pelts [29,32,33]. In terms of local context, the overlap in haplotypes 9 and 13 between the two archaeological sites (figure 3) suggests the persistence of some mitochondrial lineages through time in the Seaside, OR area. Palmrose and Par-Tee are close geographically and in age so these overlaps are consistent with local sea otter hunting.

Further investigation is required to increase the archaeological, historical and modern mitogenome sample size from locations throughout their former range (especially from BC and Washington). Overall, the genomic results support our hypothesis of greater haplotype diversity in the archaeological populations.

(c) Implications for reintroduction

Prior to sea otter extirpation, the Oregon coast apparently served as a transitional zone between southern and northern phenotypes [42–44] and possibly haplotypes [16,39], and could serve a similar function in the present. The historical samples from Oregon, Washington and BC represent the end of the fur trade (approx. 1850–1900), just prior to extirpation [14,52]. The historical Amchitka Island sea otters (1949–1962 AD) were used for reintroductions to southeast Alaska, BC and Washington in the 1960s-on [25]; our results, therefore, probably reflect current populations in the northern regions. We are, therefore, able to examine the genetic landscape both before and after reintroduction and contextualize the Oregon sea otters therein.

A variety of factors may have contributed to the failed Oregon sea otter reintroductions in the 1970s [25], including the possibility that California (rather than Alaska) sea otters would have been a better stock source [39]. Our results indicate that the picture is more complicated: northern sea otters are closer to the archaeological and historical Oregon sea otters analysed in this study, probably reflecting the northern location of Palmrose and Par-Tee in Oregon. In comparison, the Oregon sea otters used by Valentine *et al*. [39] came from archaeological sites along the central and southern Oregon coast. Valentine *et al*. did find two northern haplotypes in their archaeological Oregon sea otters, while the Oregon sea otters analysed in this study did not match California haplotypes. Larson *et al*. [16] analysed archaeological Oregon sea otters from throughout the Oregon coast and found gene flow occurred both to the south and the north. Taken together, these results strongly point to genetic variation along a latitudinal cline.

In addition to geographical variability, there is a methodological explanation for the difference in results: Valentine *et al*. [39] used short D-loop sequences following standard protocols at the time, while the analysis presented here used the complete mitogenome. We trimmed the mitogenomes to the 222 bp used by Valentine *et al*. [39] and performed a new network analysis (electronic supplementary material, figure S1): half of the Oregon sea otters grouped with northern haplotypes, but the other half shifted to group with the modern California haplotypes. The resulting haplotype difference demonstrates the value of whole mitogenomes in fully assessing diversity beyond the D-loop and expanding interpretations based on shorter sequences (further discussion in the electronic supplementary material).

We conclude that while reintroducing primarily California sea otters to the regions analysed by Valentine *et al*. [39] in southern Oregon may yield better results, we contend that future Oregon reintroduction efforts should include sea otters from Washington, BC and Alaska populations, especially reintroductions occurring on the northern half of the Oregon coast. Including both northern and southern sea otter populations will reflect the hypothesized pre-fur trade hybridization zone between groups [16,42–44], and reintroducing sea otters from multiple source populations may also promote increased genetic diversity [16].

(d) Novel methodological approach

Dental calculus from historical museum specimens, in combination with archaeological dentine/bone analysis, can provide a window into past genetic diversity of extirpated populations. This study is a novel demonstration of the feasibility of successfully extracting and amplifying complete mitogenomes using dental calculus from non-human mammals. Previous analysis has been limited by the number of specimens available for sampling, and future analyses can include archaeological, historical, and modern sea otters from additional locations and in larger numbers by using dental calculus to increase the resolution of genetic patterns. Nuclear genome data (including from dental calculus) may be used to identify specific adaptations [15,78], and other applied methods such as isotopic analyses should be performed to establish past ecological contexts [79,80]. While human dental calculus has been used in genomic analyses as a source of endogenous DNA [61,62], non-human dental calculus has not been used for this purpose and our study is unique in employing this method. Given the degraded nature of sea otter DNA recovered from dental calculus from recent specimens (*ca* 1983) in this study (electronic supplementary material, figures S4 and S5) and others [64], we recommend using protocols designed specifically for ancient DNA when collecting calculus from skeletonized museum specimens. This approach has great potential to provide genetic data from rare museum specimens without destroying the host tissue in the service of an integrated approach to conservation challenges in the present.

Data accessibility. Raw sequence data is available through the NCBI SRA under BioProject accession PRJNA550086. Consensus sequences and the alignment file used for analysis (ModAlign.fa) are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.djh9w0vxz [75]. Specimens analysed in this study are curated at the National Museum of Natural History in Washington, DC, the Museum of Natural and Cultural History in Eugene, OR, and the Santa Barbara Museum of Natural History, CA.

Authors' contributions. H.P.W., C.A.H. and T.C.R. conceived the study. H.P.W. and C.A.H. identified sea otter samples. H.P.W., C.A.H., R.M.A. and N.D.D. conducted the ancient DNA work. M.L.M. contributed financial support and T.C.R. sampled dental calculus. H.P.W. and C.A.H. performed data analysis and wrote the first draft of the manuscript. All authors contributed substantially to the final draft.

Competing interests. We declare we have no competing interests.

Acknowledgements. John Ossosky, Suzanne Peurach, Esther Rimer and Dave Rosenthal at the National Museum of Natural History (Washington, DC) facilitated access to teeth and sampling, as did Jon Erlandson, Pamela Endzweig and Elizabeth Kallenbach at the Museum of Natural and Cultural History (Eugene, OR), and Paul Collins at the Santa Barbara Museum of Natural History (CA). Cara Monroe and LaShanda Williams assisted with laboratory work and Tanvi Honap and Krithi Sankaranarayanan generously provided advice with data analysis. Karissa Hughes, Alexis Mychajliw, Kristen Rayfield and Robin Singleton (LMAMR) and Nelson Ting, Stephen Dueppen, Jessica Stone, Colin Brand, Diana Christie, Alexana Hickmott and Samantha Queeno (UO) provided helpful feedback. Briece Edwards shared suggestions for ethnographic data sources, and Kathy Ralls and Bob Bailey kindly provided input. We thank two anonymous, knowledgeable reviewers for their thorough and thoughtful feedback which helped to improve this manuscript. This work was supported by the University of Oklahoma, the University of Oregon Department of Anthropology Travel Funds and Cheryl Harper Fellowship, and a University of Oregon Graduate School 'Special Opps' grant.

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