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Archaeological Recovery of Late Pleistocene Hair and Environmental DNA from Interior Alaska

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ABSTRACT

Ancient hair and remnant plant DNA are important environmental proxies that preserve for millennia in specific archaeological contexts. However, recovery has been rare from late Pleistocene sites and more may be found if deliberately sought. Once discovered, singular hair fragments are not easily identified to taxa through comparative analyses and environmental DNA (eDNA) extraction can be difficult depending on preservation or contamination. In this paper, we present our methods for the combined recovery of ancient hair specimens and eDNA from sediments to improve our understanding of late Pleistocene environments from the Holzman site along Shaw Creek in interior Alaska. The approach serves as a useful case study for learning more about local environmental changes.

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Introduction

Much of what we know about the peopling of the Western Hemisphere comes from a cluster of deeply stratified archaeological sites along Shaw Creek in interior Alaska (Dilley 1998; Holmes 2001; Potter, Holmes, and Yesner 2013; Yesner 2001) including the recently discovered Holzman site (Wygal et al. 2018). The region continues to provide incontrovertible evidence for human activities and resource use going back at least 14,000 cal BP.

Mammalian hair is primarily composed of keratin, a durable protein, which accounts for its survival in some depositional contexts (Gilbert et al. 2004; Metcalfe 2018; Tridico et al. 2014). Hair also provides the opportunity for taxonomic identification through morphological analysis, measurement of stable isotope ratios for dietary and paleoenvironmental reconstruction, and potential for ancient DNA analysis (Bonnichsen et al. 2001; Metcalfe 2017; Szpak et al. 2015). When found in an archaeological context, hair can identify taxa associated with spaces used by ancient people (Prates, Ballejo, and Blasi 2016). Ancient plant and animal DNA may also survive in favourable depositional contexts as environmental DNA (eDNA) or more specifically for stratified archaeological sites as ancient sedimentary DNA or sedaDNA (Edwards 2020; Wilson 2020). Edwards (2020, 39) argues that 'it is now time to develop effective data archives for sedaDNA, refine our understanding of central issues such as taphonomy, and further expand the potential for describing, both qualitatively and quantitatively, the history of past ecosystems.' While Shaw Creek has long been known for its excellent bone and ivory preservation because of deep calcareous loess deposits (Dilley 1998), there have been no published attempts to examine its potential to preserve ancient hairs or eDNA.

In this paper, we describe the archaeological and depositional context of the late Pleistocene components from the Holzman site, along Shaw Creek. We describe excavation protocols (Figures S1–S6) and compare the effectiveness of water flotation with dry screening analyses of bulk hearth samples in the discovery of ancient hair specimens. To reconstruct the local vegetation history of the site, we applied eDNA analyses from five stratigraphic levels to test the concept that ancient DNA may be preserved in the sediment profile. The effectiveness and potential drawbacks of these approaches are discussed. Our intention is to test new analytical methods for understanding the people and paleoecology of late Pleistocene Alaska at the local level and highlight the potential for wider applicability elsewhere.

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Background

Archaeological Context

People arrived in the Americas by migrating east across the mammoth steppe through northeast Russia and Alaska (Goebel, Waters, and O'Rourke 2008; Guthrie 1982; Hopkins 1967). The earliest evidence for people in Alaska has been found overlooking the flats along Shaw Creek, a northern tributary of the middle Tanana River in interior Alaska (Holmes 2011; Potter, Holmes, and Yesner 2013, 2017). At Shaw Creek, archaeological components date at the earliest between 14,100 and 12,000 cal BP, offering evidence for the collection and use of woolly mammoth (*Mammuthus primigenius*) ivory alongside hearth and other camp features. Initial zooarchaeological analyses of osseous remains indicate consumption of migratory waterfowl (Anatidae), big game such as bison (*Bison* sp.), wapiti (*Cervus elaphus*), caribou (*Rangifer rangifer*), moose (*Alces alces*), and, in rare occasions, woolly mammoth and Pleistocene horse (*Equus lambei*) (Holmes 2001; Krasinski and Yesner 2008; Lanoë and Holmes 2016). Evidence for fish (Salmonidae) use along the middle Tanana River near Shaw Creek dates as early as 11,800 cal BP (Choy et al. 2016; Halffman et al. 2020). While much has been gained from decades of intensive investigations at these sites, the discovery of Holzman provides the opportunity to refine archaeological field methods and take a closer look through the recovery of ancient hairs from hearth features and plant DNA from the stratigraphic profile.

The late Pleistocene in interior Alaska was a period of rapid environmental and ecological transition from the late glacial through the Younger-Dryas period (*ca.* 15,000–11,800 cal BP). Soon after 15,000 cal BP, the dry steppe environment with forbs, sedges, and grasses diminished and between 14,000 and 13,000 cal BP was replaced by a shrub-tundra with an increase in birch (Bigelow and Edwards 2001) until the onset of the Younger Dryas period when a cooler and drier period fostered a return of sedges and grasses including *Artemisia* and Poaceae between 12,800 and 11,500 cal BP (Graf and Bigelow 2011, 435). Bigelow and Edwards (2001, 208) characterise the next phase by a rise in *Betula* (birch) and *Salix* (willow). Populus increased between 11,100 and 9400 cal BP (Graf and Bigelow 2011).

The middle Tanana Valley, including the Shaw Creek flats area, was ice free throughout the Last Glacial Maximum and formed a refugium for Pleistocene fauna that attracted people to cross into the Americas from Asia (Guthrie 1982; Hopkins 1967; Yesner 2007). Retreating Pleistocene glaciers expanded available territories and created foraging opportunities for people to spread south from interior Alaska into deglaciated landscapes and throughout the Western Hemisphere (Dalton et al. 2020; Potter et al. 2018; Wygal and Krasinski 2019). Several hundred years after the first signs of people in Alaska, the Clovis tradition appeared in association with woolly mammoths south of the Laurentide and Cordilleran ice sheets (Figure 1) (Haynes 2002, 2015a, 2015b; Jennings and Smallwood 2019). The timing and migratory route for the peopling of the Western Hemisphere remains the subject of intense debate (Potter et al. 2018) despite hypotheses based on paleo-genomics (Willerslev and Meltzer 2021) and questions concerning the recently discovered human footprints at White Sands, New Mexico reportedly dated 22,000 cal BP (Bennett et al. 2021).

It is within this broader context that we seek to understand the local paleoecology and archaeology of the Shaw Creek flats as a potential migratory pathway for late Pleistocene people. The Holzman archaeological site is a multicomponent site discovered in 2015 located on an east-facing low ridge overlooking Shaw Creek about a half-mile upstream from its confluence with the middle Tanana River (Wygal et al. 2018). At Holzman, five stratigraphically separated cultural components are sealed in 1.7 m of aeolian silty sand, loam, and loess (Table 1). The earliest cultural components, C4 and C5, date to the Younger Dryas and Allerød chronozones, respectively (Figure 2). The stratigraphy and cultural chronology are similar across many sites along Shaw Creek including Broken Mammoth, Mead, and Swan Point (Dilley 1998; Kielhofer et al. 2020). These sites represent the earliest undisputed archaeological evidence in the Western Hemisphere.

Holzman component 5b occurs in the sandy loam deposits at 170–165 cm below surface – at least 11 cm below component 5a. A complete mammoth tusk was recovered and its collagen was AMS dated in separate labs between 14,310–13,810 cal BP (D-AMS 018572 and BETA 465550). The tusk was found within a weak and discontinuous organic-rich stringer between fine and course grained sand beds. This stratum also produced a bison rib bone dated 13,770–13,520 cal BP, pressure flakes directly associated with charcoal (*Salix* sp.) dated 14,150–13,810 cal BP (BETA 531772), and dispersed bone and ochre fragments. Radiocarbon dates listed in Table 2 have been calibrated using OxCal v4.4.2 (Bronk Ramsey 2020) with the INTCAL20 climate curve (Reimer et al. 2020).

Component 5a contains a mammoth ivory workstation with an expedient quartz technology and two well-preserved hearths containing burnt avian remains and some charred flora. The earliest evidence for food preparation and hearth activities alongside ivory tool manufacture at Holzman has been dated

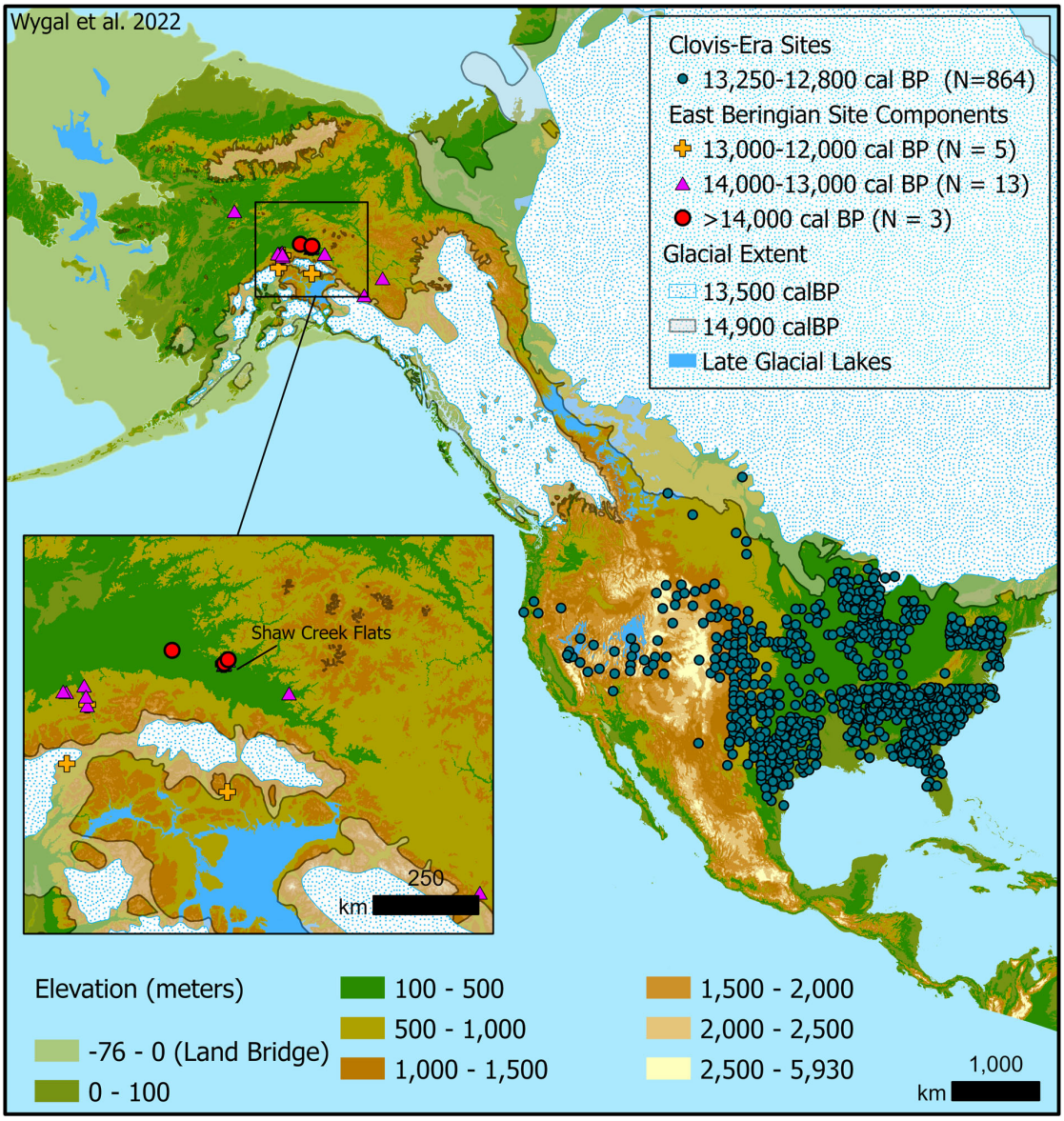

Figure 1. Extent of late Pleistocene Glaciation in North America. Ice extent after Dalton et al. (2020). Ancient Lake Lahontan and Bonneville after Duke and King (2014). Ancient Lake Atna after Wiedmer et al. (2010). Ancient lakes in Beringia after Bond (2019). Proglacial lakes in Alberta after Utting (2019; Utting and Atkinson 2019). Clovis-era sites in the lower 48 and southern Canada from Anderson and Faught (1998).

between 13,590–13,440 cal BP (BETA 531773). The oldest hair specimen (AU-19-1043) recovered thus far was found in hearth F19-02 from component 5a. Characterised by coronal 'stacked crown' scale pattern and uniserial ladder medulla with hockey puck shaped cells; this specimen is currently unidentified (Figures S7–S8).

Component 4 consists primarily of large hearth features associated with cooking activities that include scattered burned medium-large mammal bone fragments identified as *Bison* and cervid. Long bones were broken for marrow extraction and sub-component 4b contained a butchery event of at least one bison. Sub-component 4a contained multiple hearths with overlapping radiocarbon ages suggesting repeated occupation of the site, perhaps seasonally. The hearth features from component 4a have significantly higher levels of plant charcoal than hearths from component 5a. Collected from around the component 4a hearths and mixed among the fragmented fauna were five lithic tools and 64 debitage flakes of chert, siltstone, and local quartz associated with 63 gastroliths (Tables S1–S3). A fragment of *Salix-Populus* charcoal recovered from hearth F17-05 dated 11,950–11,750 cal BP (BETA 481084). A single strand of hair (AU-17-356.4) was recovered from this hearth

Table 1. Stratigraphy of the Holzman site.

Stratum	Component	Depth cm bs	10% HCL	Colour		Field Texture	Paleosol Complexes	Brief Description	Radiocarbon cal BP*
				Dry	Moist				
I	Historic	0–8	NE	10YR2/2d	10YR2/1m	duff		90% forest duff and roots	
		8–12	NE	10YR5/3d	10YR3/2m	duff and loam		organic-rich; many roots with loam matrix; hydrophobic	
II	C1	12–32	NE	7.5YR4/4d	7.5YR4/3m	very fine sand		reddish colour; micaceous-rich; red clay discontinuous lenses 7.5YR4/4, <3 mm thick; many fine rootlets	1070–930
III	C2	32–52	NE	10YR4/6d	10YR4/4m	loamy very fine sand		orangish colour; micaceous rich; well sorted; massive	
IV		52–106	NE	10YR5/3d 10YR4/4d	10YR4/3m	fine sandy loam		mottled light grey/orange; well sorted; massive, sterile of artifacts	
V	C3	106–116	SL	10YR5/3d	10YR4/3m	silt loam	Upper	Series of organic-rich layers separated by mottled loam with CaCO3 stringers	10380–10210
	C4a	116–122	SL	10YR4/3d	10YR4/2m	loam			
	C4a	122–126	ST	10YR4/3d	10YR4/2m	loam	Middle-1st		11950–11190
	C4b	126–132	SL	10YR3/3d	10YR4/1m	loam	Middle-2nd		12620–11950
		132–138	ST	10YR4/3d	10YR4/2m	very fine sandy loam			
	C5a	138–144	SL	10YR4/2d	10YR3/2m	silt loam	Lower-1st		13790–12830
		144–162	ST	2.5Y4/3d	2.5Y4/3m	loamy fine sand			
VI	C5b	162–172	NE		2.5YR4/2m	loamy fine sand	Lower-2nd	light grey; massive, thin/faint discontinuous paleosol at 165–170 cm bs	
		172–173	NE		2.5YR4/3m	medium sand		laminated sand lens,	14150–13520
		173–335	NE		2.5YR4/2m	loamy fine sand		light grey; massive	
VII		335				Gneiss, schist, quartz veins		bedrock	

Note: Sedimentology by T. Wriston.

*Calibrated with OxCal v4.4.2 (Bronk Ramsey 2020) using the IntCal20 climate curve (Reimer et al. 2020) from bone and charcoal samples but do not include dates from ivory. See Table 2 for more radiocarbon information. Depth below surface varies slightly across the site. Information recorded from the north profile of the 2015 test unit.

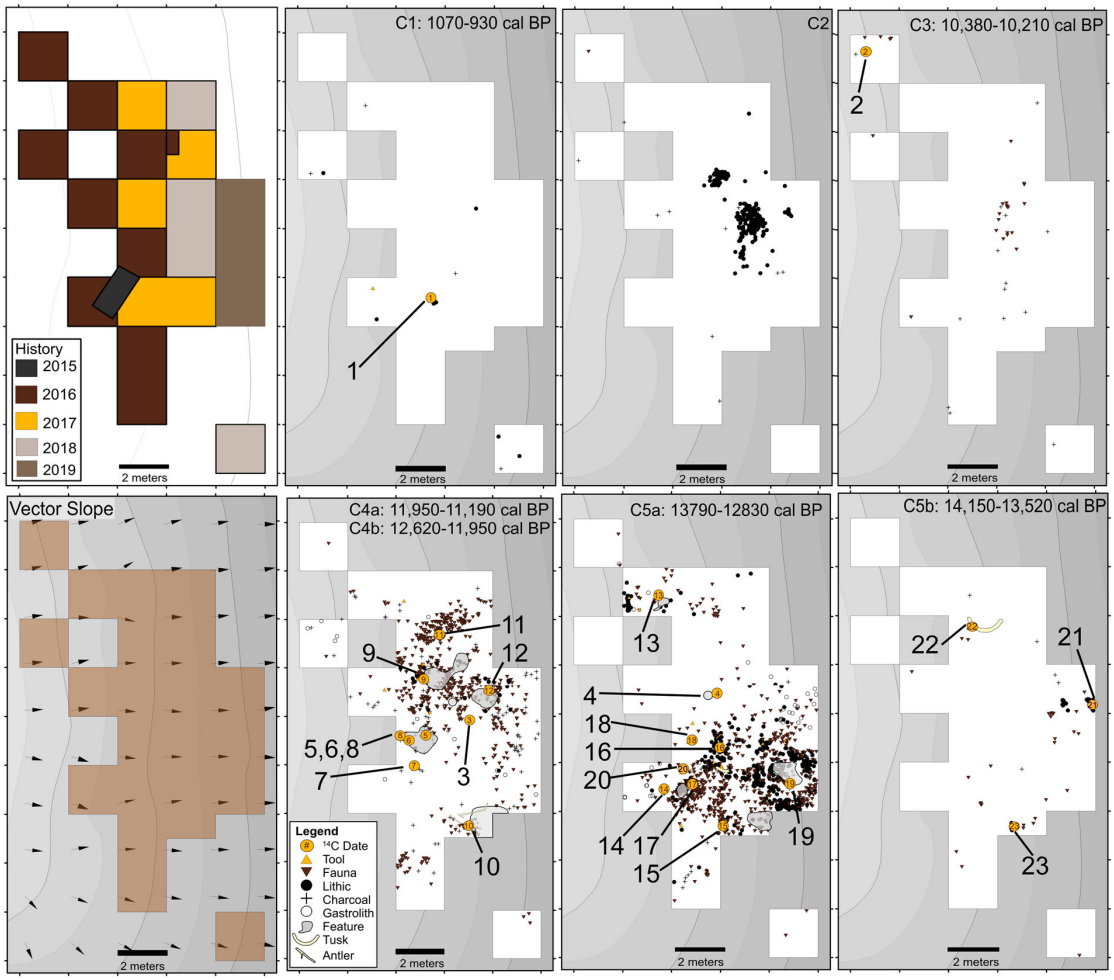

Figure 2. *In-situ* artifact distribution and slope vector maps depicting excavation history for six cultural components at Holzman. Radiocarbon dates and association contexts are identified here by the Map # from Table 2. Map #9 from C4a was from charcoal recovered in hearth F17-05 containing hair AU-17-365.4. Map #8 from C4a was on charcoal in hearth F16-01 containing possible ungulate hair AU-16-169. Map #19 in C5a is from hearth F19-02 that yielded hair AU-19-1043.

and is evaluated more thoroughly later in this paper. An adjacent hearth (F16-01) dated between 11,950–11,400 cal BP (D-AMS 019822) also yielded an uncarbonized hair (AU-16-169) fragment discovered under the microscope. Too small for formal analysis, its size and barely visible lattice medulla suggest it could be from an ungulate.

Depositional Context

The sequence of aeolian loess, loam, and sand deposited at Shaw Creek is nearly uniform (between 3.5 and 4.5 m in depth) throughout the middle Tanana Valley as correlated between several key sites including Broken Mammoth, Mead, and Swan Point (Dilley 1998; Gilbert 2011; Kielhofer et al. 2020; Lanoë et al. 2018). The local bedrock consists of gneiss and schist with extensive veins of quartz. Aeolian sand and calcareous silt from the glacially-fed Tanana River cap the surrounding hills of the Yukon-Tanana Uplands (Péwé 1965). At Holzman, the upper 170 cm of stratigraphy consists of six distinct units averaging 10 cm of accumulation every 800 years, although the Pleistocene sands accumulated more rapidly than the Holocene loess sequences. While there is no evidence for cryoturbation, the Holzman deposits do freeze during winter months favouring excellent preservation.

Radiocarbon dates establish a consistent chronology of *in situ* cultural components including well-preserved fauna, lithics, and hearth features in separately sealed and distinct layers (Figure 3). Cultural activities enhanced a series of natural organic-rich paleosol 'stringers' throughout the valley (Gilbert 2011; Kielhofer et al. 2020). A refit analysis provided no evidence for vertical mixing between components 4 and 5 (Holt 2019) and lithic raw material composition from each component indicates tool stone use differed between the components (Table S1–S2).

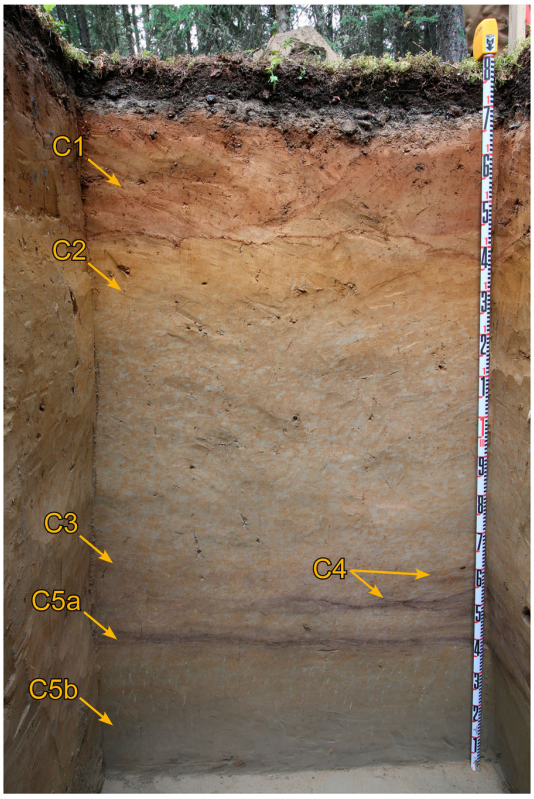

Figure 3. Stratigraphy and artifact components from north profile of 2015 test unit at the Holzman site. Additional profiles available in Supplemental Data Report. Photo by T. Wriston (after Wygal et al. 2022, 69 Fig. 4).

Methods and Results

Excavation Methodology

Excavation of the Holzman site was organised by 2 × 2 m units sub-divided by 1 × 1 m quadrants and 50 × 50 cm sub-quadrants. A TopCon G235 electronic theodolite recorded all *in situ* proveniences and site topography. All features were drawn, measured, and photographed. Sediment was screened through 1/8-inch wire mesh maintaining 5-cm vertical control. Hearth and other organic-rich features were bisected and bulk samples of fill collected in aluminum foil packets sealed in large 4 ml artifact bags then air dried slowly in a controlled environment. The meticulous excavation allowed for three-dimensional spatial and slope vector analyses of all artifacts and dispersed charcoal by stratigraphic and cultural levels.

To reduce cross-component and external contamination, we developed an aDNA excavation protocol based on consultation with the Trace and Ancient sedimentary DNA (TrEnD) Laboratory at Curtin University, Australia (Dan Werndly, personal comm.). The excavation team wore nitrile gloves, surgical masks, and shoe covers for excavations below 100 cm and replaced these multiple times per day. Clean Visqueen© sheets separated excavators and tools from contact with cultural deposits and reduced external contamination. All tools including buckets and screens were sterilised with 15% Clorox© solution twice per day and between cultural components and each excavation unit was assigned specific screening stations to avoid cross contamination. A canopy of translucent Visqueen tarps diverted forest debris and rainfall from the excavation and screen areas (Figure S6). While tedious, the DNA protocol reduced the risk of external contamination of transfer DNA by the excavation team.

Environmental DNA

Environmental DNA may be recovered from various sources including air, ice, fresh or salt water, and ancient sediments to provide broad characteristics of plant and animal ecosystems (Ficetola et al. 2008). Scientists have moved beyond the proof of concept and regularly use eDNA to evaluate the method's ability to characterise species presence within specific ecosystems (Stewart 2019). Recovery of ancient sedimentary DNA or sedaDNA is an eDNA technique applied to more thoroughly understand ecosystem changes from stratified contexts, such as archaeological or paleontological sites. Recent examples of ancient eDNA recovery and analysis in archaeological research include studies on the resilience of arctic flora to climate change from Svalbard, Sweden (Alsos et al. 2016) and lake eDNA has revealed a long livestock farming history from near Lake Anterne in the French Alps (Giguet-Covex et al. 2014). Shirazi et al. (2019) extracted ancient DNA going back 50,000 years ago from Klondike permafrost cores to broadly outline plant and animal changes through time. In neighbouring Alaska, eDNA work has been limited save for an unpublished preliminary lake core study from the Tanana valley near Shaw Creek (Edwards et al. 2021). At the Holzman site, we sampled six late Pleistocene and early Holocene sediments for study at the Paleogenomics Laboratory, University of California Santa Cruz. Detailed methodological descriptions for the plant biodiversity analysis are expanded upon in the Supplemental Data Report.

Initial eDNA analyses from Strata IV-VI at Holzman, containing cultural components 4 and 5, provides a relative abundance of plant groups observed for the lower strata (Figure 4, Table S3) and is consistent with previous pollen studies for interior Alaska (Ager 1975; Bigelow and Edwards 2001; Bigelow and Powers 2001). PCR replicates detected on average 14 plant taxa and found the DNA profile from component 5b Stratum VI samples (dating to ~14,000 cal BP) were dominated by forbs, with 94% of the reads assigned to the Rosaceae family. The most recent three samples from Strata IV and V (encasing

Table 2. Radiocarbon data from the Holzman site.

Map#	Lab #	14C BP	1σ	calBP 2σ	Component	Context/Association	Material dated
1	BETA 479326	1110	30	1070–930	1	small hearth F17-01, FCR with charcoal	charcoal, Picea sp.
2	BETA 494405	9120	30	10,380–10,210	3	dispersed bone fragment	bone, caribou metapodial
3	BETA 508997	9820	30	11,270–11,190	4a	adjacent to hearth features F17-04 & 17-05	bone, large mammal
4	BETA 479325	10030	30	11,740–11,340	4a	postmold intrusive into C5a F17-07	charcoal, Salix
5	D-AMS 019823	10061	54	11,820–11,330	4a	hearth F16-01	charcoal, Populous-Salix
6	BETA 465548	10070	40	11,820–11,390	4a	hearth F16-01, base of feature	charcoal, Populous-Salix
7	BETA 415563	10090	30	11,820–11,400	4a	hearth F16-01	charcoal, Populous-Salix
8	D-AMS 019822	10149	54	11,950–11,400	4a	hearth F16-01, ungulate hair AU-16-169	charcoal, Salix
9	BETA 481084	10200	30	11,950–11,750	4a	hearth F17-05, charcoal, hair AU-17-356.4	charcoal, Populous-Salix
10	BETA 548587	10340	30	12,470–11,950	4b	antler cache F19-01	charcoal, Populus-Salix
11	D-AMS 019819	10356	44	12,480–11,970	4b	bone cluster F16-02	bone, bison
12	BETA 508358	10450	40	12,620–12,100	4b	charcoal from hearth F18-03	charcoal, Populus
13	BETA 465551	11030	40	13,080–12,830	5a	greasy residue with some bone F16-03	bone collagen, large mammal
14	D-AMS 014248	11417	37	13,340–13,170	5a	anvil activity area, ivory reduction	bone collagen, large mammal
15	BETA 548588	11500	40	13,470–13,300	5a	lithic reduction area	bone collagen, bird
16	D-AMS 019818	11538	81	13,590–13,240	5a	ivory reduction area	bone collagen, large mammal
17	BETA 479328	11540	30	13,480–13,320	5a	hearth F17-06, ivory reduction area	bone collagen, large mammal
18	BETA 465549	11600	30	13,580–13,350	5a	fractured mammal remains	collagen, mammoth ivory rod (tool)
19	BETA 531773	11640	30	13,590–13,440	5a	hearth F19-02, hair AU-19-1043	burnt twig, Betulaceae
20	D-AMS 016636	11827	34	13,790–13,590	5a	ivory reduction area	collagen, mammoth ivory
21	BETA 531771	11800	30	13,770–13,520	5b	sand paleosol, lithic flakes, ochre flecks	collagen, bison rib
22	D-AMS 018572	12137	47	14,150–13,810	5b	sand paleosol	collagen, mammoth tusk AU-16-635
23	BETA 531772	12140	40	14,150–13,810	5b	sand paleosol, attached to a flake	charcoal, Populus-Salix group
22	BETA 465550	12200	40	14,310–14,020	5b	sand paleosol	collagen, mammoth tusk AU-16-635

Note: Radiocarbon dates calibrated using OxCal v4.4.2 (Bronk Ramsey 2020) with the INTCAL20 climate curve (Reimer et al. 2020). The Figure 2 site component map depicts the location of Map # listed here. Detailed stratigraphic descriptions are Table 1.

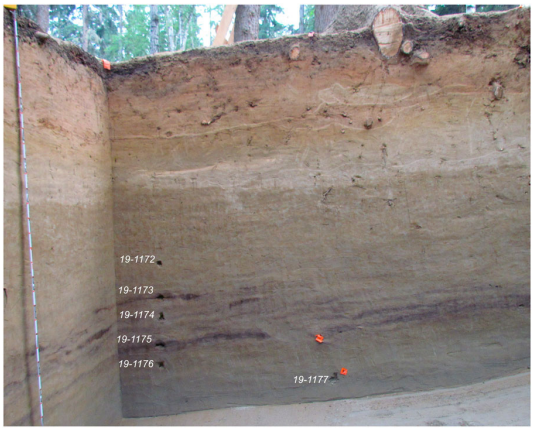

Figure 4. Location of eDNA samples taken from Strata IV-VI at the Holzman site in 2019.

components 4 and 3) dated to circa 12,500 and 9000 cal BP, respectively, were dominated by woody plants including willow (*Salix sp.)*, birch (*Betula* sp.), and spruce (*Picea* sp.) (Figure 5). While differences in deposition rates exist, degradation of DNA, and biases in eDNA processing mean that the proportion of reads assigned to taxa does not represent the precise relative abundance of plants within an ecosystem (Figure S11). These data nonetheless reflect a pronounced shift in flora from an arid steppe environment with limited vegetation to herbaceous shrubland in interior Alaska. The results of our initial eDNA sediment analysis further demonstrate the stratigraphic integrity of late Pleistocene deposits at the Holzman site and provide a site-specific environmental history. We anticipate further studies will continue to improve the clarity with which to understand these past ecosystems.

Recovery of Hair from Hearth Fill

In the lab, fill from several hearth features was systematically weighed and split using a riffle sampler. Half of the samples were subjected to flotation and the other half were dry-screened. A 2 mm sieve was placed above the 500-micron sieve to collect and size-sort specimens. In the case of dry-screening, soil that passed through the 500-micron sieve was bagged as 'residue' and was not scanned under the microscope. During flotation, materials that passed through the 500-micron screen were lost in overflow. All materials collected in the sieves (flotation light and heavy fractions; dry-screening >500 micron and >2 mm fractions) were scanned at low power under a Leica stereo microscope with standard halogen light followed by ultraviolet light. Two ultraviolet flashlights were used: a one-watt flashlight (Inova X5MT-WUVT) with a 365–400 nm wavelength output, and a higher power 15-watt flashlight (Alonefire SV-13) with a 365 nm wavelength output.

During the air drying stage, a single strand of hair (AU-17-356.4) from C4 was observed embedded in an intact sample of hearth fill (F17-05) prior to dry sieving (Figures 6, S9–S10). The hearth was located at the centre of a dense artifact scatter 4.5 m in diameter consisting mostly of broken mammal bone but also containing lithic artifact debris. Viewed under a dissecting microscope at 10–40x prior to removal from the matrix, the hair was uncharred, consistent with secondary deposition after the burning event. Tiny burnt bone fragments were observed and wood charcoal collected from the hearth fill and identified as *Salix-Populus*.

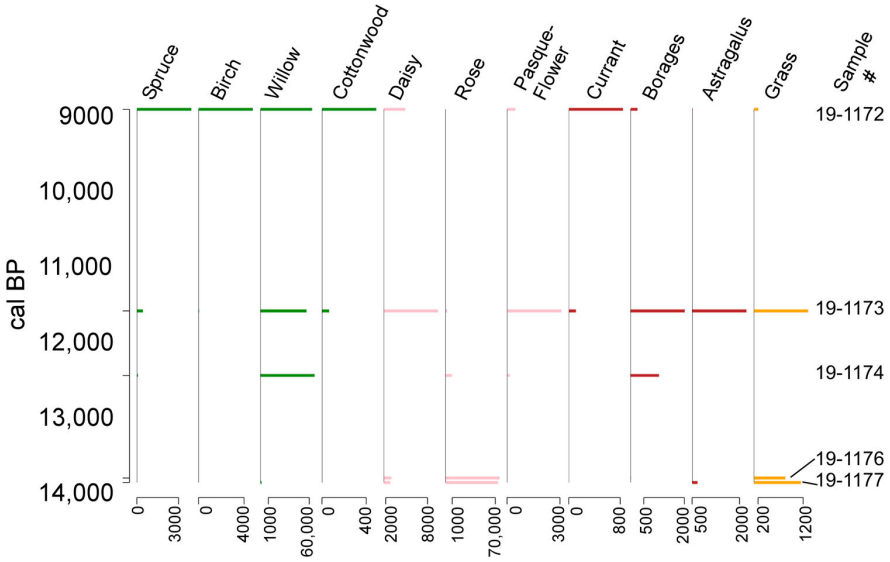

Figure 5. Average sequence count across PCR replicates for ancient plant DNA from Holzman.

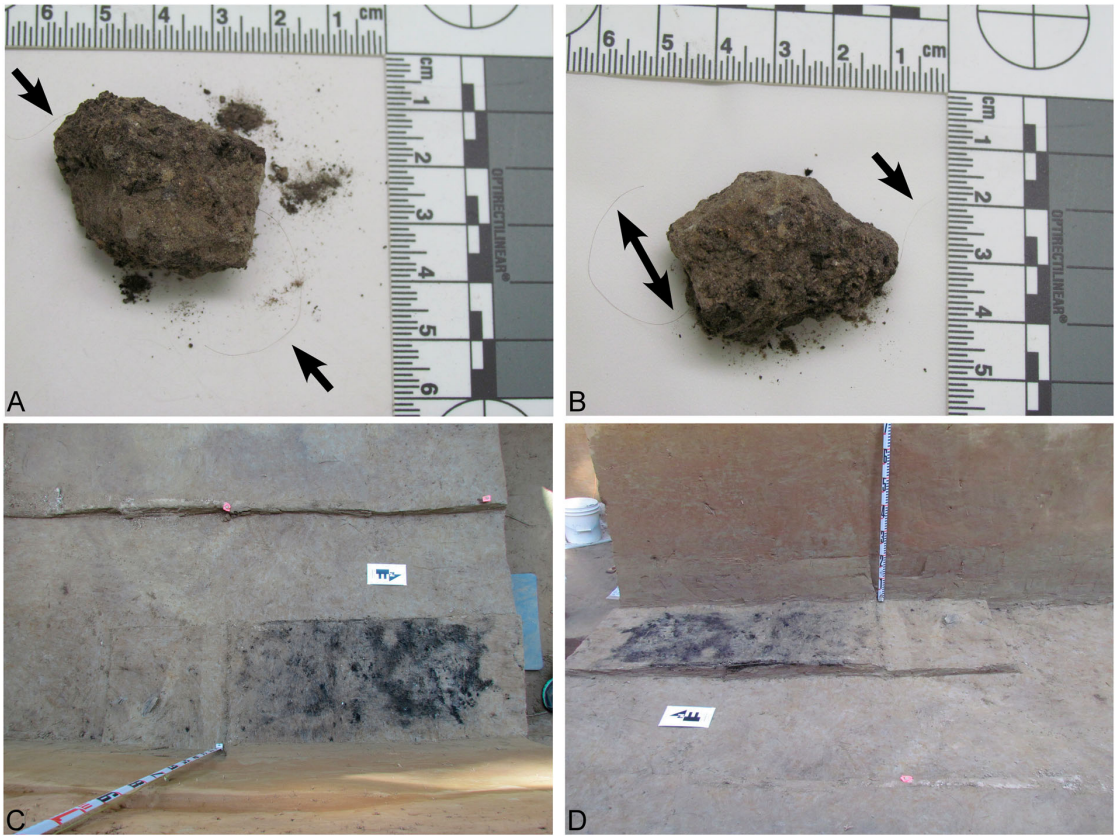

Figure 6. Hair (AU-17-356.4) from component 4a of the Holzman site was recovered in an intact bulk sample of charcoal-rich hearth (F17-05) sealed within loess deposition. **A**. Ancient hair strand found encased in bulk hearth matrix prior to dry sieving. **B**. Alternate angle of hair running through hearth matrix. Arrows point to hair strand passing through either side of the hearth matrix. **C**. Profile view with cross-sectioned hearth feature *in situ*. **D**. Alternate angle of cross-sectioned hearth feature in which the bulk sample was recovered.

Hair Comparative Analysis

The hair specimen AU-17-356.4 was initially inspected, photographed, and its length measured using metric graph paper. For microscopic examination, the hair was placed in a temporary wet mount solution of buffered glycerol and covered with a 44 × 22 mm glass slip. Following observations and photography, the hair was removed from the wet mount and rinsed in 91% isopropyl alcohol. Prior to removal from the collodion, the hair was observed under both transmitted and incident light sources without a cover slip. No attempt was made to cross-section the hair because this type of slide preparation would be destructive to the specimen. A second analyst examined the hair under reflected and transmitted light using a low-powered dissecting microscope at 10–45x magnification. Since the hair appeared dirty, it was gently wiped with a paintbrush soaked in methanol to remove surface contaminants. The hair was then temporarily mounted in Cargille© Type A immersion oil (refractive index: 1.5150) and examined under transmitted light at 100–400x magnification. After examination, the specimen was rinsed with acetone. Cuticular scale casts were made by placing the hair on a thin film of nail polish. After casting, the hair was again cleaned by rinsing in acetone in preparation for subsequent aDNA analysis.

For taxonomic identification, the hair was compared with physical reference samples of extinct and extant taxa, including woolly mammoth (*Mammuthus primigenius*), American bison (*Bison bison)*, steppe bison (*Bison priscus*), muskox (*Ovibos moschatus*), modern horse (*Equus caballus*), Pleistocene horse (*Equus lambei*), donkey (*Equus asinus*) and human (*Homo sapiens*) hair. Online resources, including the Alaska Fur Project (Carrlee 2011) and published descriptions were consulted (Chernova, Boeskorov, and Protopopov 2015; Metcalfe 2018; Moore, Spence, and Dugnolle 1974; Spasskaya, Chernova, and Ibraev 2012; Tridico et al. 2014).

Hair specimen AU-17-356.4 was 8.8 cm long and ranged from 40–50 µm in diameter over the length of the shaft. The hair profile was slightly wavy (curved) and the colour was brown with no banding (Figures S9–S10). The root was elongated and only slightly wider than the shaft, with a blunt but not bulbous

end. There was no follicular tissue attached. The medulla was absent throughout the length of the shaft. Longitudinal fissures and pits were present on the surface of the hair, suggesting it had experienced mechanical damage and/or fungal invasion. The cuticle was heavily damaged but, where visible, the cuticular scales were rippled with moderate spacing and an irregular wave/mosaic pattern.

A robust literature pertinent to the macroscopic and microscopic features of extinct and extant mammal hair exists and can support taxonomic identification based on a combination of features, including size, pigmentation, shaft curvature, cuticular scale pattern, and features of the medulla (Carrlee 2011; Chernova et al. 2016; Chernova and Kirillova 2013; Kosintsev and Plasteeva 2011; Metcalfe 2018; Moore, Spence, and Dugnolle 1974; Spasskaya, Chernova, and Ibraev 2012; Tridico et al. 2014). However, hair identification is complex since hair features can vary considerably within a species and individual. Faunal remains from component 4 indicate the presence of bison and caribou.

Many features of the Holzman hair clearly differentiate it from cervid (moose, wapiti, caribou, deer) hair, including its macroscopic appearance (lack of undulations or banding), small diameter, lack of medulla, and rippled irregular wave cuticle pattern. The features of the hair are also not characteristic of wolf, lynx, fox, wolverine, bear, or Beringian lion. The root of the Holzman hair is highly dissimilar to human hair roots and the cuticular scale pattern further suggests that the hair is not human. The cuticular scale pattern is less regular than in horse hairs. All of these taxa can reasonably be excluded as matches for the component 4 hair specimen (Figure 7).

The macroscopic and microscopic features of the Holzman hair are most consistent with either mammoth or bison guard or underhair. Bison underhair typically has a single, relatively wide, continuous or sometimes fragmented medulla, whereas mammoth underhair typically lack a medulla or have a very narrow fragmented medulla. However, both bison and mammoth underhair can lack a medulla. In a comprehensive study of *Bison priscus* hairs, Chernova and Kirillova (2013) observed a medulla in all underhairs (referred to as 'zigzag' and 'woolly' hairs) with shaft diameters >45 μm, but a lack of medulla in some hairs with diameters of < 45 μm. At 40–50 μm diameter, the Holzman hair falls at the cusp of these two intervals, but its maximum diameter falls within the interval expected to contain a medulla. Published images of *Bison priscus* 'woolly hairs' in the <45 μm range had smoother scale margins and more regular scale wave/mosaic patterns than the Holzman hair (Chernova and Kirillova 2013). The *Bison priscus* 'zigzag hairs' had scale patterns more similar to the Holzman hair, but unlike the Holzman hair, all zigzag hairs had either a continuous or fragmental medulla (Chernova and Kirillova 2013). In comparison, all mammoth hairs with 40–50 μm shaft diameters had an absent medulla. Mammoth underhairs have cuticular scale patterns that are typically more irregular than those of bison (e.g. Chernova, Kirillova, Boeskorov, and Shidlovskiy 2015; Tridico et al. 2014).

Unfortunately, few published images of mammoth underhair cuticular scale patterns are available and comparative analyses of underhairs do not typically yield definitive identification to species. Tridico et al. (2014) describe 'finer underhairs' of woolly mammoths and rhinos as having a broad petal pattern, with rounded non-prominent edges, but no images were provided. This does not describe the scale pattern of the Holzman hair. However, it is not clear if their description was meant to describe the whole range of mammoth underhairs (with 20–100 μm shaft diameters) or only those with the smallest diameters, since very fine hairs (<30 μm shaft diameters) can have petal-like patterns in a variety of mammal species. Overall, the comparison of cuticular scale patterns was inconclusive in differentiating between bison and mammoth. While it is true that *Mammuthus primigenius* hairs of this size lack a medulla whereas *Bison priscus* hairs with 45–50 μm diameters generally have a pronounced medulla, it is not possible to distinguish the Holzman hair from these two possibilities through comparative analyses alone.

aDNA Analysis of Hair

We attempted to more definitively identify the hair specimen AU-17-356.4 through two ancient DNA extracts (MOE166 and BAN039) following the protocol described by Dabney and Meyer (2019) with modifications to reduce contamination (Korlević and Meyer 2019). We converted these extracts into double-stranded DNA sequencing libraries following Kapp, Green, and Shapiro (2021). We then cleaned the libraries with SPRI magnetic beads in 18% PEG-8000 solution and sequenced each on an illumina MiSeq using v3 chemistry and 2 × 75 reads. We performed all molecular protocols preceding PCR in the Paleogenomics Laboratory at the University of California Santa Cruz. We also used Blastn v 2.6.0, together with MEGAN v6.21.2 (Huson et al. 2007) to determine sequence matches between adapter-trimmed filtered reads and the GenBank v226 database.

To analyse the taxonomic position of the hair source, we analysed the first extract by processing the sequencing reads using standard protocols (Vershinina et al. 2020; Vershinina et al. 2021). For MOE166, we mapped the merged quality-filtered reads on a variety of reference genomes; however, the results did not indicate DNA library affinity to

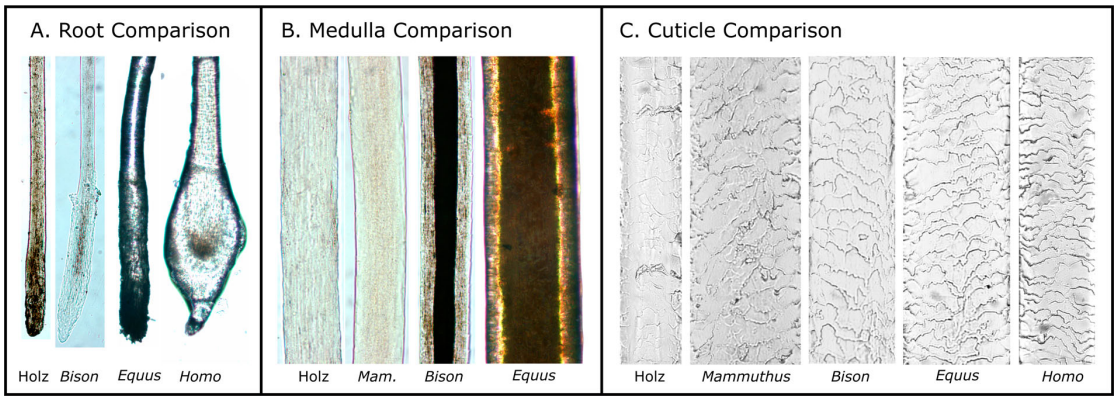

Figure 7. Selected features of the Holzman hair AU-17-356.4 in comparison to hairs of reference taxa. **A**. Roots of the Holzman hair, a modern wood bison (*Bison bison athabascae*), a modern horse (*Equus caballus*), and a Caucasian human (*Homo sapiens*), all at 100x magnification. **B**. Medulla comparison for the Holzman hair, a woolly mammoth (*Mammuthus primigenius*), a modern wood bison, and a donkey (*Equus asinus*), all at 400x magnification. **C**. Cuticular scale patterns of the Holzman hair, a woolly mammoth, a steppe bison (*Bison priscus*), a Caucasian human, and a Pleistocene horse (*Equus lambei*), all at 400x magnification. We emphasize that this figure does not depict the full range of variation in any of these hair features, as described in the text.

any particular organism or reference and suggested human contamination. Next, we prepared the second DNA extract, merged the read data (MOE166 and BAN039) and mapped it to the human reference genome, hg38. In addition to nuclear genome analysis, we used the online github DNA post processing program[1] (Vershinina et al. 2020) to assemble the mitochondrial genome recovered in libraries MOE166 combined with BAN039, using NC 012920 (rCRS) as a seed for reference-free MIA v1.0 assembly. We then ran the assembly through HaploGrep 2.0 (Weissensteiner et al. 2016) to determine the haplogroup of the assembled mitochondrial genome.

Our attempts to identify the hair from aDNA extraction were inconclusive but do provide some insight into the source of hair specimen AU-17-356.4. We received 914,546 read pairs for MOE166, of which 362,005 passed filtering and quality control (Figure S12). We merged reads following the best practices of aDNA analysis and mapped them to a panel of 20 reference genomes (Table S4). We found that *Bison* and human reference genomes had top hits among the panel (1037 for *Bison* and 14,258 reads for human, respectively). However, a Blastn v.2.6.0 search indicated human as the most likely source of molecules in the MOE166 library (Figure S13). While DNA in the sample was fragmented, we did not find evidence of cytosine deamination in the DNA damage profile, suggesting the human DNA molecules were modern (Figures S14–S15).

We made an additional DNA extract to further investigate the possibility of human DNA present in the hair. After merging read data from MOE166 and BAN039, we recovered 1,389,290 reads in total, 781,146 of which we retained after quality filtering. Of these, a total of 39,302 (merged and unmerged, non-duplicated) reads mapped to the human reference genome hg38. MapDamage profile of those mapped reads still did not indicate any signs of cytosine deamination, characteristic of aDNA. Our mitochondrial genome assembly produced a complete mitochondrial genome of the combined MOE166-BAN039 sample. HaploGrep 2.0 determined HV0 as the most likely haplogroup of the assembled mitogenome. HV0, occurs at the highest percentages in Western Europe, indicates either potential European origin of the sample or contamination from a modern source. Contamination is a pervasive problem in ancient DNA samples and extreme care is needed to produce reliable results from post-mortem damaged DNA. We should note that, prior to arriving at the Paleogenomics Lab, the hair was analysed in three different labs, none of which were sterile. Thus, we consider contamination by modern human DNA to be very likely. From the results described above, we conclude that the majority of viable hits were from Bison and the second majority from cow; although, even these levels were too low for a confident identification.

While it may not be possible to verify the source of the hair specimen AU-17-356.4 through current aDNA or comparative analyses alone (the single hair was deemed too small for ZooMS), the combined evidence suggests the most likely taxon is *Bison*. The hair features were consistent with either bison or mammoth but the DNA results show almost 5x the reads to bison as to mammoth and about 2x as many reads as any non-human animal. Although we were not able to date the hair directly (once again, too small), we have established that it is ancient based on the level of degradation and its secure archaeological context (discovered within the intact matrix of a bulk hearth sample). Therefore, the human DNA reads from the hair were likely the result of inadvertent modern contamination of an ancient bison hair.

Discussion

Paleoenvironmental reconstructions of interior Alaska are essential to understanding the phases of transformation from the arid steppe ecosystem of the late Pleistocene to the mesic boreal forest that arose during the middle Holocene. For twenty years, two studies on pollen frequencies from pond cores (Bigelow and Edwards 2001; Bigelow and Powers 2001) have provided archaeologists with a coarse-grained view of vegetation history for interior Alaska that have been 'difficult to interpret' at the local site level (Bigelow and Powers 2001, 171). Traditional proxy records such as ancient hair and newer methods like eDNA recovery can assist in understanding ancient environmental changes at the hyper-local level of an individual archaeological site.

In this study, we successfully sequenced eDNA plant profiles from five out of six sediments sampled from the Holzman archaeological site at Shaw Creek (see Figures 4 and 5). The method provides evidence for the presence of specific plant communities at the sub-component level. At the Holzman site, we identify the presence of daisy, rose, astragaulus, and grasses in the oldest cultural component 5b generally dated 13,600–14,100 cal BP (samples 19–1176 & 19–1177). Our attempt to sample plant eDNA was unsuccessful from a particularly organic rich portion of cultural component 5a, dated 13,000–13,600 (19–1175). Willow, borages, and some rose was detected near an otherwise poorly preserved caribou antler cache in the component 4b sample (19–1174) dated between 12,470–11,950 cal BP. Component 4a, dated between 11,950–11,190 cal BP, contained the most hearth features documented at the site and yielded eDNA from willow, cottonwood, daisy, pasqueflower, borages, astragalus, and grass with a trace amount of rose and currant (sample 19–1173). Sterile loess of approximately 9000 cal BP was also sampled (19–1172) and contained spruce, birch, willow, cottonwood, daisy, and currant, with smaller amounts of pasqueflower, borages, and grasses.

Ancient hairs of mummified animals, including mammoth, bison, horse, woolly rhinoceros, and cave lion have been routinely reported throughout Beringia (Siberia, Alaska, and Yukon Territory) and have been studied extensively (Chernova, Boeskorov, and Protopopov 2015; Chernova, Kirillova, Boeskorov, and Shidlovskiy 2015; Chernova, Kirillova, Boeskorov, Shidlovskiy, and Kabilov 2015; Harington 1980; Harington and Egglestone-Stott 1996; Tridico et al. 2014; Zazula et al. 2009). Because organics often preserve well in the acid-neutral loess sediments of interior Alaska, the discovery of ancient hairs in this archaeological context is not a great surprise but recovery of hair at archaeological sites in this region remains rare. This highlights the need for archaeomicroscopy to be more fully integrated in archaeological research.

The recovery of ancient hairs was more successful through dry sieving than through water flotation. Furthermore, the careful macroscopic and microscopic analyses of bulk soil and hearth samples collected in a sterile setting can provide other valuable proxy evidence of plants and animals represented at specific archaeological sites. Researchers should take advantage of low power magnification and UV illumination as well as more powerful forensic-type alternate light sources and other tools (e.g. high-powered petrographic microscopes and SEM) to identify and recover ancient hairs and this process can easily be applied in the field or laboratory. These methods are not new to archaeology but have not been regularly applied in late Pleistocene contexts.

Single strands of hair present unique problems for archaeologists. Comparative analyses can be informative but are not always conclusive, and site taphonomy and contexts must be carefully described. It is important to assess the degree of degradation on hair specimens to determine if they are modern contaminants or from the prehistoric era. In cases such as these, it would be most useful to obtain a direct radiocarbon date on ancient hair samples to rule out any possibility of modern contaminants. While some radiocarbon labs may have the capacity to date individual hair proteins, because their mass is so small, this option is not widely available commercially.

New opportunities also present themselves in the form of advances in eDNA research from archaeological sites, especially for key sites in Alaska where the frozen silts and clay depositional environments favour DNA preservation. According to Edwards (2020, 40),

> the most promising sediments are cold or frozen, anaerobic, around neutral pH and/or dry (as in cave deposits). Clay-silt particles bind to DNA fragments and tend to reduce enzymatic activity in the process, though recovery of eDNA may also be successful from organic-rich sediments.

Because contamination introduced by the excavator and/or sample processor can be easily introduced, strict DNA protocols are essential to reduce inadvertent contamination. Minor adjustments to excavation methods are logistically difficult with large crews and add cost (i.e. masks, gloves, and decontamination procedures) to already expensive endeavours, but these greatly reduce the introduction of external DNA contamination, particularly from meal residues transferred by touching artifacts directly.

There is also a need for archaeologists, aDNA geneticists, and hair experts to work collaboratively to develop laboratory protocols for hair identification that will minimise the opportunity for contamination, allowing for microscopic examination of hairs while

not precluding the extraction of aDNA. Experimental studies may be needed to determine appropriate sterilisation techniques for laboratory equipment and to identify techniques for hair examination (e.g. embedding and casting methods) that are compatible with aDNA or ZooMS analyses. These protocols should be considered prior to undertaking excavations with the specific intention of recovering and analysing hair and ancient eDNA.

Conclusion

In this paper, we describe the implementation of protocols applied at the Holzman site for the discovery of ancient hair and remnant plant eDNA from late Pleistocene archaeological contexts in interior Alaska. Our intention is to draw attention to important trace evidence with promise to provide detailed paleoecology and environmental histories at a local level, despite the challenges. Once discovered, single hair fragments are not easily identified to taxa through comparative analyses and DNA extraction can be difficult depending on preservation levels and external contamination. Nevertheless, these methods can provide detailed paleoecology and environmental histories at a local level.

Note

1. https://github.com/Paleogenomics/DNA-Post-Processing/blob/master/mito_assembly_pipeline.sh (Vershinina et al. 2020).

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