

Boise State University

ScholarWorks

Anthropology Faculty Publications and
Presentations

Department of Anthropology

4-2017

Determining the Population Affinity of an Unprovenienced Human Skull for Repatriation

Jennifer K. Watkins

Boise State University

Samantha H. Blatt

Boise State University

Cynthia A. Bradbury

Boise State University

Gordon A. Alanko

Boise State University

Matthew J. Kohn

Boise State University

See next page for additional authors

Publication Information

Watkins, Jennifer K.; Blatt, Samantha H.; Bradbury, Cynthia A.; Alanko, Gordon A.; Kohn, Matthew J.; Lytle, Marion L.; and Butt, Darryl P. (2017). "Determining the Population Affinity of an Unprovenienced Human Skull for Repatriation". *Journal of Archaeological Science: Reports*, 12, 384-394. <https://doi.org/10.1016/j.jasrep.2017.02.006>



This document was originally published by Elsevier in *Journal of Archaeological Science: Reports*. This work is provided under a Creative Commons NonCommercial-NoDerivs 4.0 license. Details regarding the use of this work can be found at: <http://creativecommons.org/licenses/by-nc-nd/4.0/>. doi: 10.1016/j.jasrep.2017.02.006

Authors

Jennifer K. Watkins, Samantha H. Blatt, Cynthia A. Bradbury, Gordon A. Alanko, Matthew J. Kohn, Marion L. Lytle, and Darryl P. Butt



Determining the population affinity of an unprovenienced human skull for repatriation



Jennifer K. Watkins^{a,d,*}, Samantha H. Blatt^b, Cynthia A. Bradbury^b, Gordon A. Alanko^{a,d}, Matthew J. Kohn^c, Marion L. Lytle^c, Joanna Taylor^e, Deborah Lacroix^e, Maria A. Nieves-Colón^f, Anne C. Stone^{f,g}, Darryl P. Butt^{a,d,h}

^a Department of Materials Science & Engineering, Boise State University, 1910 University Dr., Boise, ID 83725, USA

^b Department of Anthropology, Boise State University, 1910 University Dr., Boise, ID 83725, USA

^c Department of Geosciences, Boise State University, 1910 University Dr., Boise, ID 83725, USA

^d Center for Advanced Energy Studies, Boise State University, 995 University Blvd., Idaho Falls, ID 83401, USA

^e Center for Advanced Energy Studies, University of Idaho, 995 University Blvd., Idaho Falls, ID 83401, USA

^f School of Human Evolution and Social Change, Arizona State University, 900 Cady Mall, Tempe, AZ 85287, USA

^g Center for Bioarchaeological Research, Arizona State University, 900 Cady Mall, Tempe, AZ 85287, USA

^h College of Mines and Earth Sciences, University of Utah, Salt Lake City, UT 84112, USA

ARTICLE INFO

Article history:

Received 28 July 2016

Received in revised form 5 February 2017

Accepted 5 February 2017

Available online xxxx

Keywords:

Provenience

Repatriation

Skull

Cinnabar

mtDNA

Cranial deformation

NAGPRA

ABSTRACT

An archaeological assessment was carried out on an unprovenienced human skull recovered in eastern Idaho, exhibiting cranial deformation and peri-mortem application of a red pigment. A combination of scanning electron microscopy (SEM), X-ray fluorescence (XRF), and energy-dispersive X-ray spectroscopy (EDS) identified the major and trace elements present in the red pigment as natural cinnabar. Carbon and oxygen stable isotopes from teeth and bone suggest a mostly C3 plant-based diet with subsidiary consumption of salmon or marine resources, and a regional geographic transition between early life and late adulthood. Radiocarbon dating determined the approximate age of the skull to be between 600 and 700 years old, and ancient mitochondrial DNA assessment identified characteristics of haplogroup B, one of four major Native American mitochondrial DNA lineages, which is consistent with the osteological analyses.

© 2017 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Despite the legal consequences of ignoring the Native American Graves Protection and Repatriation Act (NAGPRA) and regional laws in regards to collection and selling human skeletal remains, illicit trafficking of human remains is not abated. Therefore, law enforcement and state archaeologists must have effective means of identifying population affinity of remains with unknown or lost provenience in order to resolve such cases. Though ancestry can be estimated, comparative osteological databases can be insufficient when identifying remains to a specific region or culture, as great variability in regions and through time exist (Seidemann et al., 2009). This is confounded for forensic and archaeological remains that lack context due to collection, trafficking, and sale of remains. This ambiguity often leaves bioarchaeologists unable to conclusively assign specific population affinity for repatriation. This paper reports the results of a multidisciplinary analysis of an

unusual human skull with unknown provenience and unknown affinity. It combines traditional and alternative methods and denotes the importance of exhausting multiple means to narrow down skeletal affinity in a post-NAGPRA environment in which descendent population-bioarchaeologist relations are ever so fragile and important to maintain.

In November 2013, a human skull with very limited and vague origins came into the possession of the Boise State University, Anthropology Department from the Idaho State Historic Preservation Office, Western Repository. Some basic observations, such as soil in the nasal aperture and eye orbits, suggested that it had been interred. The skull was particularly notable both for its cranial deformation and for a reddish to brownish pigment applied over much of the vault and face (Fig. 1 and Fig. 2). Prior to this, the remains had been curated by the Archaeological Survey of Idaho, Northern Repository (ASINR) located at the University of Idaho, Alfred W. Bowers Laboratory of Anthropology (Moscow, ID). The accompanying report stated the remains were collected from an unknown location near Rigby, Jefferson County, in eastern Idaho and then donated to ASINR from the local sheriff's office sometime prior to 1976. This report stated that the cranium and mandible were brought to ASINR with materials from site 10CW1, but the

* Corresponding author at: Department of Materials Science & Engineering, Boise State University, 1910 University Dr., Boise, ID 83725, USA.

E-mail address: jenniferwatkins1@boisestate.edu (J.K. Watkins).

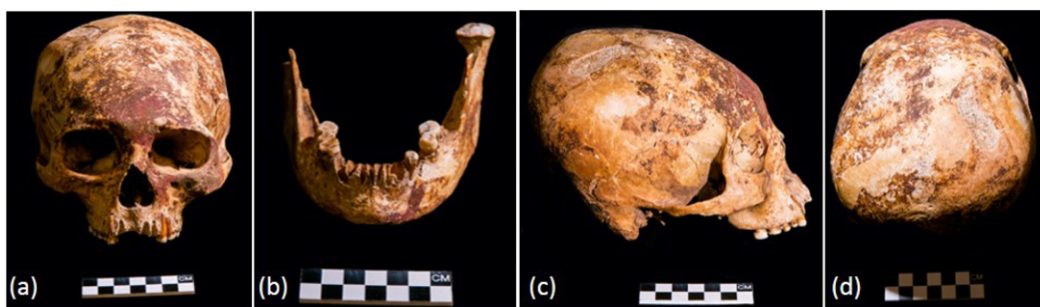


Fig. 1. Photographs for the (a) cranium and mandible of F112413; the red pigment was applied near the bregma, at the top of the cranium, down to glabella on the frontal, to both maxillae, and across the anterior body of the mandible. Fronto-occipital cranial deformation is depicted in the lateral view (c) and superior view (d).

remains might not actually derive from that site. The cultural remains from 10CW1 had been repatriated years ago without the cranium because it was not believed to be part of that collection, yet specific knowledge of its origins had been lost (Idaho State University staff, pers. comm.). At this time the skull was brought to Boise State University, it was assigned the case number F112413.

Although the provenience of the skull was ambiguous and incomplete, its association with other Native American artifacts and pre-modern appearance suggested it may also be of Native American origin, thus calling into question the applicability of NAGPRA. Given these suspicions and the drive to repatriate remains, an investigation into the temporal and geographic backgrounds, and biological affinity of the skull was undertaken, with permission from ASIWR and state archaeologists, using multidisciplinary methods. Here, we describe the complementary results of these different methods, which narrow the origins of these otherwise enigmatic materials, with the purpose of repatriation.

2. Background and scientific rationale

In bioarchaeological and forensic anthropology studies, no one approach is singularly diagnostic of the origin of the individual (particularly when only a skull remains, as in this case), but in summation can help narrow possible geographic locations. Thus, several independent datasets were collected. Specifically, we employed several complementary methods, including analysis of skeletal morphology, pigments, hair, mitochondrial DNA (mtDNA) and stable isotopes, as well as dating the bone using ^{14}C . The following sections provide background information and rationale to explain what information we can gain from, as well as

limitations of, each technique we employed with the ultimate objective of ascribing affinity for repatriation.

2.1. Systematics of cranial deformation

As mentioned, skull F112413 appeared to be artificially deformed/modified. This observation played a role in decisions made subsequently about the methods used in this report. Artificial cranial deformation serves as a cultural artifact that preserves better than other forms of body modification and can serve to help reconstruction of social systems, stratification, migrations, and ethnic identity of past peoples. Intentional cranial deformation has been practiced on nearly every continent of the world and in different historical contexts, and may have originated as early as 15,000 years ago (Anton, 1989; Brown, 1981; Dingwall, 1931). Ethnographic accounts have indicated that some cultures considered deformation a mark of beauty (Boas, 1890), while in others it was a symbol of elevated status (Ortner, 2003), or a form of body decoration marking group affiliation (Dingwall, 1931; Gerszten, 1993).

Deformation can also be unintentional, such as the flattening of the occipital bone through the use of a cradleboard (when the infant frequently sleeps on a hardened surface) seen in many North American populations (Kohn et al., 1995). In both intentional and unintentional cases, deformation begins prior to cranial fusion when the bones of the cranium are more malleable. Ethnographic and ethno-historic sources indicate that intentional or artificial deformation often begins within a few days of birth with a flattening apparatus being used for 6 months to 5 years (O'Loughlin, 2004). Groups practicing deformation

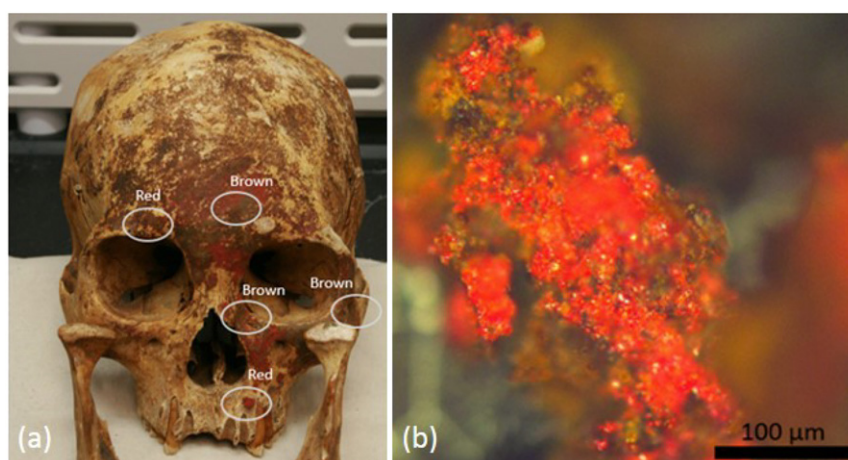


Fig. 2. (a) Photograph of the skull F112413 with markers indicating from where the pigment samples were taken. (b) Red pigment sample under optical microscopy at 20× magnification.

would either strap hard, flat devices like boards, to both the front and back of the infant's head, or wrap the infant's head with tight bandages, like cords, permanently altering the head shape.

Although as many as 16 types of deformation have been described, only two forms are common: annular (or circumferential) and fronto-occipital (or antero-posterior) deformation (Anton, 1989; Anton and Weinstein, 1999; Clark et al., 2007; Gerszten, 1993). Annular deformation is associated with binding the cranium and compressing it cylindrically, limiting growth medio-laterally and resulting in increased cranial length and a decrease in breadth. Annular deformation has been cited among the Kwakiutl and Nootka (prehistoric Northwest coast), and some populations from Peru and Arawe (Clark et al., 2007; Gerszten, 1993). Fronto-occipital deformation involves binding the cranium to a flat surface, compressing the cranium antero-posteriorly and constricting growth between the frontal and occipital. Fronto-occipital deformation is characterized by decreased cranial length, increased cranial breadth, and a flattening of the front and occipital bones resulting in the bulging of the parietal bones. This type of deformation has been cited from the Ancon, Peru, Makapuan, Hawaii, American Southwest (e.g. Hopi), and the Songish from the Pacific Northwest, among others, and is typically the result of cradleboarding (Clark et al., 2007; Gerszten, 1993). With all this in mind, the cranial deformation in skull F112413 could not serve as a cultural, regional, or temporal indicator for repatriation.

Though cranial metrics are routinely applied for inventory and descriptive purposes, due to potential bias in metric analyses from the cranial modification, they were not used in assessing ancestral affinity. Notwithstanding such issues, the Amerind sample in the Forensic Data Bank, which is often used to estimate ancestry from craniometrics, consists only of modern Native Americans from the southwestern United States as it is, which may not be representative of prehistoric or pre-modern indigenous American groups from other regions. It was felt that craniometrics results using these standard data banks as reference could only be presented with great caution and would only confound interpretation in this case and, thus further investigation was warranted.

2.2. Pigment and hair analysis

Many different ancient and prehistoric cultures, separated by both geographical region and time, have utilized red pigments like those observed on skull F112413, most commonly ochre or iron oxides (Fe₂O₃). Indeed, red ochre was widespread in funerary rituals during the Late Archaic period in North America (Wreschner et al., 1980). In the case of the present skull, the red and brown pigments adhering to the cranium and mandible were not staining the bone and were not consistent with red ochre. The potential for an unusual – and geographically distinctive – pigment prompted us to focus mineralogical and chemical analysis on this pigment. Identification of the hairs could link the skull to resource use of other mammals, e.g. bison, deer/elk, small mammals, etc. that might be geographically distinctive.

2.3. Mitochondrial DNA analysis

Mitochondrial DNA can be used to estimate population affinity through identification of geographically diagnostic mtDNA lineages known as haplogroups. Our primary objective was to obtain the complete mtDNA genome of the individual by extracting and quantifying genetic material from the remains. Our specific goals were to build sequencing libraries, recover endogenous ancient DNA through target enrichment capture, and then sequence the mtDNA genome with next generation Illumina MiSeq technology. Mitochondria are the energy producing organelles of eukaryotic cells. Each cell typically contains several hundred mitochondria, and each mitochondrion has several copies of a circular genome approximately 16,500 base pairs (bp) in length. Due to the high copy number of mitochondrial DNA found per

eukaryotic cell, it is more likely to survive over time than nuclear DNA in skeletal remains from archaeological contexts. MtDNA is inherited solely through the female line and does not recombine (Giles et al., 1980). Therefore, mitochondrial lineages are informative of female population history. These lineages can be identified through screening of diagnostic polymorphisms such as the 9 base pair (bp) deletion. Human groups across the world carry characteristic mtDNA lineages which have arisen through migration and regional demographic histories. Native American mtDNA lineages cluster into four major groups defined by the 9 bp deletion or certain restriction sites, as well as by specific mutations in the mtDNA genome (Schurr et al., 1990). Most of these haplogroups are also found in Asian populations but they are typically rare.

2.4. Stable isotopes

Tooth enamel and bone $\delta^{18}\text{O}$ values can also provide information about origins like the methods above because they correlate with $\delta^{18}\text{O}$ values in surface water, such as streams, rivers, or lakes, which varies geographically (Chenery et al., 2012; Daux et al., 2008; Luz et al., 1984). After development, tooth enamel remains unchanged throughout life except for mechanical abrasion and is resistant to diagenetic changes (Koch et al., 1997). Bioapatite (approximately $\text{Ca}_{4.5}[(\text{PO}_4)_{2.7}(\text{HPO}_4)_{0.2}(\text{CO}_3)_{0.3}(\text{OH})_{0.5}]$) is compositionally most similar to hydroxylapatite and is the main component of enamel (Pasteris et al., 2004). Either the phosphate (PO₄) or carbonate (CO₃) component can be analyzed for isotopic composition. Carbonate component analysis results in values for both carbon and oxygen stable isotopes, which are proxies for diet and ingested water respectively (Koch, 1998; Kohn and Cerling, 2002).

Bone bioapatite has a similar chemical composition to that of enamel, albeit lacking substantial OH (Pasteris et al., 2004), and also provides carbon and oxygen stable isotope compositions. Unlike enamel, it re-equilibrates continuously through an individual's lifetime, and sometimes post-burial. Tooth enamel, therefore, indicates food and water sources early in life (because enamel is unchanging after growth), while bone represent more ante-mortem sources. Because jaw bone remodels ~90% over 5 years (Huja et al., 2006), the mandible isotopic composition that we measured reflects the last several years that the individual was alive. We do not think the bone has undergone substantial isotopic alteration post-burial, in part because diagenetic alteration commonly destroys collagen, whereas sufficient collagen was retrieved from the same bone for ¹⁴C analysis. Moreover, studies that compare completely recrystallized fossil bone vs. enamel show compositions within ~2% (Kohn et al., 2015; Kohn and Law, 2006), so a small amount of alteration should not bias compositions substantially. Comparing bone and enamel compositions allows for detection of geographic migration after enamel growth has completed and therefore, could be used to narrow regional origins.

Diet in the form of vegetation is reflected in carbon isotope values ($\delta^{13}\text{C}$). Plants are separated into two dominant groups, C3 and C4, based on photosynthetic pathways (Fig. 3). C3 plants typically range from –30 to –25‰ (V-PDB), but can span <–35‰ (rainforest) to almost –20‰ (Atacama desert) depending on local conditions, especially rainfall (Kohn, 2010). C4 plant compositions range between ≈–10 and ≈–14‰ (Cerling et al., 1997). Tooth isotope compositions parallel these differences in habitat but with higher $\delta^{13}\text{C}$ values. A fractionation of ≈13‰ between enamel and diet (see review of Sandberg et al., 2012) implies that plant compositions should be 13‰ lower than bioapatite compositions. In addition, when using archaeological bioapatite to calculate apparent modern plant compositions (for comparison to modern datasets whose ecologies are known), an additional ≈1.5‰ correction should also be applied to account for fossil fuel burning over the last

¹ Delta notation for oxygen is given by: $\delta^{18}\text{O} = 1000 \times \left[\frac{^{18}\text{O}_{\text{sample}}}{^{18}\text{O}_{\text{standard}}} - \frac{^{18}\text{O}_{\text{sample}}}{^{18}\text{O}_{\text{standard}}} \right]$ and analogously for carbon.

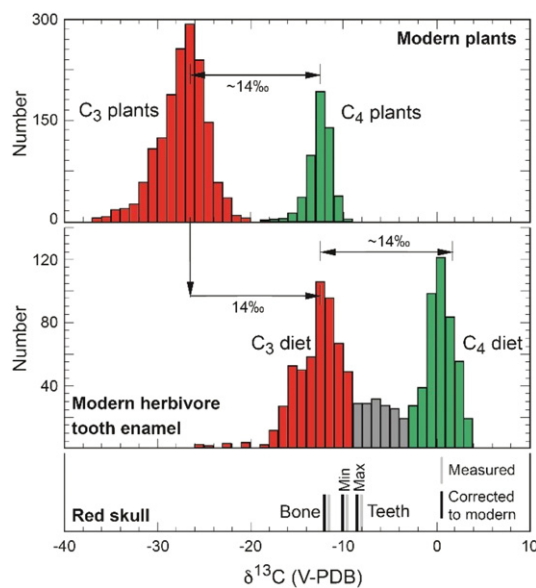


Fig. 3. The relationship between carbon isotope values of modern C3 and C4 plants and modern herbivore enamel (see Kohn, 2010; Kohn and Cerling, 2002). The skull tooth carbon isotope values are at the maximum limit of C3 plants, likely reflecting slight consumption of either C4 sources or possibly fish or shellfish.

≈ 200 years (Bauska et al., 2015; Friedli et al., 1986). Thus, an archaeological composition of -10‰ would imply equivalent modern plant compositions of -24.5‰ ($= -10\text{‰} - 13\text{‰} - 1.5\text{‰}$). Consumption of relatively high $\delta^{13}\text{C}$ salmon (c. -17 to -22‰ ; e.g., Satterfield and Finney, 2002) or especially marine foods (c. -11 to -15‰ ; Schoeninger and DeNiro, 1984) would increase $\delta^{13}\text{C}$ compared to a C3-only diet, and cause an underestimate of the ratio of C3 to C4 plants in an individual's diet. Conversely, consumption of terrestrial meat would likely reduce $\delta^{13}\text{C}$ values relative to a purely vegetarian diet (Clementz et al., 2009), and cause an overestimate of the ratio of C3 to C4 plants in an individual's diet.

Precipitation $\delta^{18}\text{O}$ values in temperate areas are controlled by distance from the source, temperature, and altitude. In Idaho, precipitation derives mainly from winter sources originating directly west in the North Pacific. Summers are dry. The winter snowpack generally exhibits low $\delta^{18}\text{O}$ values and its melt supplies streams, rivers, and lakes throughout the year. Summer aridity increases evaporation of surface water, increasing summer $\delta^{18}\text{O}$ values. Cooler temperatures and/or increasing altitudes correlate with decreased $\delta^{18}\text{O}$ (Dansgaard, 1964). Average modern Idaho surface water oxygen isotope values are $\approx -16\text{‰}$ (V-SMOW; Kendall and Coplen, 2001). Similarly, these regional values can help pinpoint geographic origin for the unprovenanced skull.

3. Materials and methods

3.1. Radiocarbon dating

Collagen was separated using bone solvent extractions (due to unknown adherents) from the condyle of the right mandible. Radiocarbon dates were determined using accelerator mass spectrometry (AMS) measurements (Beta Analytic Radiocarbon Dating Laboratory; Florida, USA; Beta-393782; November 17, 2014). The sample's $^{14}\text{C}/^{13}\text{C}$ was measured relative to $^{14}\text{C}/^{13}\text{C}$ in Oxalic Acid II (NIST-4990C) in one of four in-house stage accelerator mass spectrometers. Three quality assurance samples were measured along with unknowns. Results for the QA samples fell, as required, within expectations of known values prior to accepting the results for all samples. $\delta^{13}\text{C}_{\text{coll}}$ was obtained by oxidizing a small portion of the extract in an elemental analyzer connected directly to an isotope ratio mass spectrometer (IRMS). $\delta^{13}\text{C}_{\text{coll}}$ is reported

relative to VPDB. More detailed information on the radiocarbon dating methods is available in the supplementary content.

3.2. Skeletal analysis

The materials used in this report consist of a single skull (F112413) and the adhering pigment and hairs. The skull was macroscopically observed and standard anthroposcopic methods were applied to estimate ancestry (Buikstra and Ubelaker, 1994), age (Buikstra and Ubelaker, 1994; Gruspier and Mullen, 1991; Mann et al., 1987; Meindl and Lovejoy, 1985), and sex from the skull (France, 1998; Krogman, 1962; Walker, 2008). Craniometrics were avoided for ancestry estimation due to bias from vault modification (Fig. 1c–d). The skull has been considered to be the most accurate skeletal indicator of ancestry. Finite scaling work (Kohn et al., 1995) suggested that facial alterations due to cranial deformation were rare among Hopi and the only significant differences were in the slightly higher and narrower faces of individuals with deformed vaults. When cradleboarding produced fronto-occipital flattening, such modifications did not impact the face and cranial base and did not add bias to analysis of determining biological distance between populations. Therefore, estimation of ancestry from facial morphology non-metrics in this specimen is assumed to be unbiased despite cranial vault deformation. In contrast, craniometrics methods (for discriminant functions) for ancestry estimation were deemed too biased due to the deformation to warrant application.

3.3. Pigment and hair analysis

We used optical microscopy, scanning electron microscopy (SEM), and energy dispersive spectroscopy (EDS) to identify the red and brown pigments. A sample of red pigment with one of the hair strands embedded within it was imaged via bright field optical microscopy and SEM (Fig. 7). We especially wished to confirm that the pigment was not simply a modern, commercial paint. Using a dental pick, pigment samples were removed by gently scraping from the red and brown areas on the skull (Fig. 2a) and stored in small glass vials. Samples were affixed to an aluminum holder using carbon tape and examined via optical microscopy (Olympus BX51 with U-CMAD3 ColorView camera and analysis imager software; Fig. 2b), manual scanning electron microscopy (SEM) for microstructure and linked energy dispersive spectroscopy (EDS) for chemical composition (Hitachi 3400-N-II).

To help constrain a geological source of the colorant from trace element concentrations (Erlandson et al., 1999), three pigment samples were analyzed using a Horiba micro X-ray fluorescence XGT-7200 analytical microscope with Microanalysis Suite-Issue 17B, XGT-7200 Suite version 1.94 integrated analysis software. The same samples analyzed via SEM, EDS, and XRF were submitted for more sensitive analysis via laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS). A Thermo Electron X-Series II quadrupole mass spectrometer coupled with a New Wave UP 213 nm frequency Nd:YAG laser was used. More detailed information regarding these methods is available in the supplementary content.

Hairs embedded in the pigment and adhering to the top of the skull were collected and sent for blind comparative analysis and identification at the Department of Art Conservation Studies at the University of Delaware. Four different samples were isolated for slide preparation for optical analysis.

3.4. mtDNA analysis

Sampling and ancient DNA extractions were conducted at the Arizona State University Ancient DNA Laboratory, a Class 10,000 clean-room facility. Four extractions total were attempted, two on a lower third molar, one on calculus and one on an upper right second molar. Considerable difficulty was encountered in obtaining a non-contaminated sample. Ultimately, the amount of recovered endogenous DNA was

insufficient to build complex sequencing libraries, so target enrichment capture and sequencing were not attempted. Because of poor DNA preservation, identification of the individual's mtDNA lineage relied on polymerase chain reaction (PCR) data from amplification of the 9 bp deletion region of the mtDNA genome (Pääbo, 1989). Sample preparation and elimination of surface contaminants and inhibitors followed standard methods (Gilbert et al., 2006; Rohland and Hofreiter, 2007; Schuenemann et al., 2011). DNA extraction methods optimized for recovery of short, degraded DNA fragments from ancient teeth and calculus were used (Dabney et al., 2013; Warinner et al., 2014). More detailed descriptions of the methods used are available in the supplementary content published electronically along with this paper.

3.5. Stable isotope analysis

Six mandibular teeth from skull F112413 were analyzed to determine their carbon and oxygen stable isotope composition. The lower left canine, right and left lower first premolars, right lower second molar, and right and left lower third molars were loose but not able to be removed from the mandible without further destruction. The enamel was slightly discolored, and heavily worn or chipped post-mortem in most cases. Powdered enamel samples, removed with a Dremel, weighing 3 mg or less were reacted at 70 °C in a He gas-purged borosilicate exetainer with 0.05 mL of concentrated phosphoric acid for the carbonate standards and 0.1 mL for the bioapatite until completely dissolved (approximately 24 h). Evolved carbon dioxide samples were analyzed using continuous flow isotope ratio mass spectrometry (CF-IRMS) on a Thermo Delta V Plus IRMS with a Gasbench II headspace sampler. Data are reported in standard permil (‰) notation, relative to V-PDB (carbon isotopes) and V-SMOW (oxygen isotopes). More detailed information regarding this method is available in the supplementary content.

4. Results

4.1. Radiocarbon dating

The results provided for Beta-393782 indicate a conventional radiocarbon age of 580 ± 30 BP ($^{13}\text{C}/^{12}\text{C}$ ratio of $-14.5\text{‰} \pm 1\sigma$) for skull F112413. The INTCAL13 database (Reimer et al., 2013) provides calibration curve intercepts at 1330, 1340, and 1395 CE and a date range of 1300–1415 CE for the skull at 95% confidence. Osteological observations, such as soil coloration and dental wear, are consistent with the remains being archaeological.

4.2. Skeletal analysis

The F112413 remains consist of a well preserved cranium and mandible (Fig. 1a–b). Most teeth were missing post-mortem and the lower left 1st molar was lost pre-mortem. The severity of the dental attrition of remaining dentition is not consistent with modern diet and suggests a pre-modern or archaeological temporal affiliation. The skull exhibits soil staining and was not bleached or professionally curated. No postcranial remains are present. The coloration and adherence of small amounts of sediment in the cavities suggest that the individual had been buried in soil, but the length of interment and whether it was the primary or secondary means of mortuary treatment is unclear. Along the sagittal suture, coarse dark-colored hairs adhered to the vault. Hairs also adhered to red and brown pigments on the face.

The cranial vault is flattened on the occipital and frontal bones, forcing the parietal bones to bulge medially, known as fronto-occipital flattening. The deformation is asymmetrical as the left parietal from the superior view is more posteriorly pronounced than the right parietal. Combined, the deformation results in a cranial vault which appears taller, yet shortened from anterior to posterior.

Unfortunately, the cranium and dentition in isolation are poor indicators for age-at-death estimation. When considering the morphological impact of the deformation process as well as unknown behavioral impacts, it is highly presumptuous to estimate age in this case. Nevertheless, observations were made to see if the estimation from the original ASINR report could be repeated. The skull contained only permanent dentition, including occluded third molars, and fusion of the sphenoccipital synchondrosis, all of which indicate adult maturity (Buikstra and Ubelaker, 1994). Additionally, a total of 17 sites for sutural closure of the cranium were numerically scored (Buikstra and Ubelaker, 1994). When compared to composite scores from skeletons of known-age, results indicate an age-at-death range of 45–50 years (Gruspier and Mullen, 1991; Mann et al., 1987; Meindl and Lovejoy, 1985).

Anthroposcopic traits were similarly used to assess sex. Expression of supraorbital torus, nuchal area, and supraorbital margin were all given numerical scores of 1, indicating probable female. Overall size and ruggedness of the cranium and size and projection of the mastoid process was scored as a 2, indicating likelihood of being female (Buikstra and Ubelaker, 1994). However, frontal morphology was unable to be assessed because of cranial deformation (likely obscuring natural morphology) and chin morphology was scored as 3, ambiguous. Combined, these observations indicate the individual is consistent with expressing morphologically female features (Buikstra and Ubelaker, 1994; Krogman, 1962). Sex estimation was also completed using Walker's (2008) logistic discrimination functions, which use the same 5 scored traits as Krogman (1962) and France (1998). Four of six functions indicated probable female.

Despite the fact that dental morphology is generally very useful in distinguishing population affinity, the extent of dental attrition in this specimen precluded detailed observation of this data. The teeth are very worn and many are absent. This included the incisors, so there was no way to determine if they were shovel-shaped, most frequently found among Asian or Native Americans, but there is no indication of a Carabelli's cup on any of the upper molars (frequently found among Europeans) (Edgar, 2013).

For reasons discussed previously, instead of using cranial metrics, affinity was assessed using only anthroposcopic morphology. The face of this specimen is flat and wide with rounded eye sockets. The nose has a low bridge and small spine, and the root appears tented with a flat lower border of medium width. The zygomatics display a slight malar tubercle and are flared. The palate is elliptical in shape with a relatively straight palatal suture, and the mandible is large with a blunt chin. The specimen also appeared to have a “rocker jaw” type (the ramus of the mandible is curved convexly/inferiorly, allowing it to rock back and forth on a flat surface), which is found among Native Americans as well, though in lower frequency than Polynesians (Buikstra and Ubelaker, 1994). All the features of F112413 are consistent with an individual of Asian or Native American ancestry (Buikstra and Ubelaker, 1994; Rhine, 1990).

4.3. Stable isotope analysis

The six teeth analyzed for carbon and oxygen isotopic composition had carbon isotope values grouped between -9.7‰ and -8.1‰ and oxygen isotope values between $+16.5\text{‰}$ and $+18.0\text{‰}$ (Fig. 4). The bone had significantly lower carbon and oxygen isotope values of -11.7‰ and $+12.9\text{‰}$ respectively (Fig. 4). The isotopic compositions of teeth cluster independent of bone. This difference between teeth and bone suggests either that the environment in which this individual lived changed between the time of enamel formation (sub-adult period) and a few years before death, or that the individual migrated to a different environment that was colder.

The isotope values of the right and left 3rd molars differ by 1.4‰ for carbon and 1.5‰ for oxygen, whereas left and right 1st premolars differ isotopically by only 0.6‰ ($\delta^{13}\text{C}_{\text{carb}}$) and 0.3‰ ($\delta^{18}\text{O}_{\text{carb}}$). Differences between corresponding teeth can be used to identify diagenesis

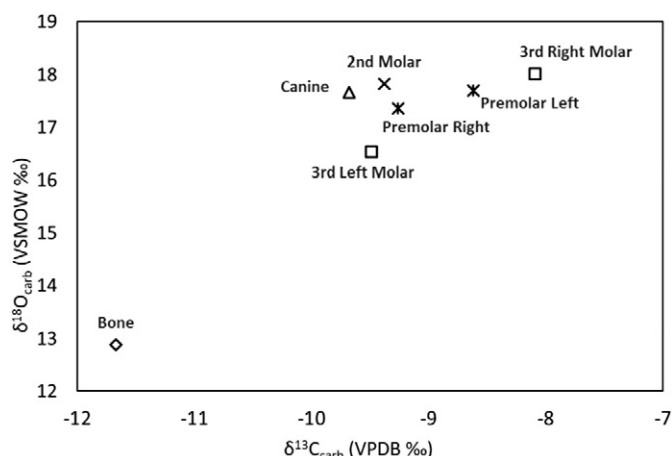


Fig. 4. Measured carbon and oxygen stable isotope values from the carbonate component of teeth and bone shows tooth values grouped together in the upper right quadrant and the bone value in the lower left quadrant. These tooth and bone values suggest this individual lived in a different environment as a child and adult.

(Prowse et al., 2007). A difference of up to 1.8‰ for 3rd molars is within the range observed for modern teeth, so the variation we measured between corresponding teeth is within the expected values for an individual. Variability in enamel formation and eruption of each tooth, coupled with seasonal and inter annual differences in isotopes, likely explain observed isotopic disparities.

The carbon isotopic values for the teeth of -9.7‰ to -8.1‰ are as high or higher than the maximum cutoff for pure C3 consumption (c. -8.5‰). The cutoff is determined from a maximum likely value of modern C3 plants of -23‰ (Kohn, 2010) increased by 1.5‰ to offset recent fossil fuel burning (Friedli et al., 1986) and by $+13\text{‰}$ for a diet-tooth isotopic offset for humans (Sandberg et al., 2012).

Oxygen isotope values for the tooth carbonate component ranged from $+16.5\text{‰}$ to $+18.0\text{‰}$. These values represent biologically processed water consumed during enamel formation which can span several years in the sub-adult period. The $\delta^{18}\text{O}_{\text{carb}}$ of the carbonate component of enamel correlates with surface or ingested water. Table 1 details the calculated values for ingested water $\delta^{18}\text{O}_{\text{IW}}$ values based on Chenery et al. (2012). The $\delta^{18}\text{O}_{\text{IW}}$ can then be directly compared with surface water. The modern Idaho surface water average based on collection of stream waters collected over ≈ 3 years is -16‰ (Kendall and Coplen, 2001). The calculated $\delta^{18}\text{O}_{\text{IW}}$ values for the skull teeth ranged from -22.3‰ to -20.0‰ (Table 1). The bone $\delta^{18}\text{O}_{\text{IW}}$ value was -28.1‰ . As determined from both tooth and bone oxygen isotopes, $\delta^{18}\text{O}_{\text{IW}}$ values are well below the average modern value for modern Idaho surface water at moderate elevations ($\approx -16\text{‰}$ at ≈ 1000 m) (Fig. 5).

4.4. Hair analysis

Two different kinds of hairs were distinguishable on F112413. Hairs 1–3 (Fig. 6) show similar scales, medullas, pigmentation, and diameters. They are light brown and very degraded; they fragmented easily and the scale patterns were hardly distinguishable. In contrast, the darkly

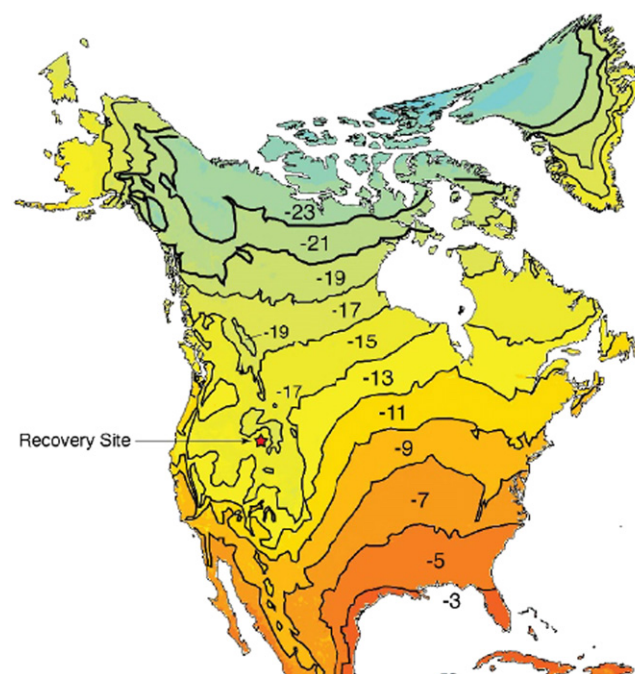


Fig. 5. Continental U.S. modern surface water (discharge) weighted mean $\delta^{18}\text{O}$ values distribution. The skull recovery site is marked with a star. Isotope and model data from Good et al. (2015).

pigmented hair sample was in good condition. Its characteristics are consistent with human hair: the scale pattern is irregular mosaic, with smooth borders near the proximal end, pigmentation is densest near the cuticle, it has an oval to circular cross-section, and narrow medulla. The hair is likely from this individual. Based on comparison with photomicrographs, the strands do not appear to be badger, bear, beaver, bison, cat, chinchilla, civet, ferret, fox, hog, horse, pine marten, stone marten, American mink, mole, mouse, muskrat, opossum, otter, rabbit or hare, rat, sable, seal, squirrel, stoat, weasel, or wolf, based on differing scale types and/or medullas (Appleyard, 1978). Instead, the hairs appear most similar to sheep's wool or the fine hairs of cattle, deer, dog, goat, marmot, or musk ox.

4.5. Pigment analysis

EDS shows the red pigment to be mercury sulfide (HgS) or cinnabar. An average Hg/S ratio for all EDS spectra indicating mercury was 1.05:1, which may indicate a loss of sulfur due to microbial activity (Minganti et al., 2007). Table 2 details these spectra according to the specific locations where they were analyzed.

Trace element analysis via XRF and LA-ICP-MS was performed on the pigment samples in the hopes of matching trace element compositions to a specific geological source for the cinnabar (Erlandson et al., 1999). Variations in apparent concentration using XRF and LA-ICP-MS likely reflect calibration differences or exact locations of analyses. XRF analysis

Table 1

Tooth formation ages and measured carbon and oxygen stable isotopic composition for the skull's teeth and bone. $\delta^{13}\text{C}_{\text{carb}}$ and $\delta^{18}\text{O}_{\text{carb}}$ are measured values from the carbonate of tooth enamel and bone. $\delta^{18}\text{O}_{\text{IW}}$ (ingested water) is a calculated value based on Chenery et al. (2012).

Sample	Initial calcification	Crown complete	Left side			Right side		
			$\delta^{13}\text{C}_{\text{carb}}$ (‰, VPDB)	$\delta^{18}\text{O}_{\text{carb}}$ (‰, VSMOW)	$\delta^{18}\text{O}_{\text{IW}}$ (‰, VSMOW)	$\delta^{13}\text{C}_{\text{carb}}$ (‰, VPDB)	$\delta^{18}\text{O}_{\text{carb}}$ (‰, VSMOW)	$\delta^{18}\text{O}_{\text{IW}}$ (‰, VSMOW)
Canine	4–5 mo	6–7 yr	−9.68	17.66	−20.6			
Premolar 1st	1.5–1.75 yr	5–6 yr	−8.62	17.70	−20.5	−9.26	17.37	−21.0
Molar 2nd	2.5–3 yr	7–9 yr				−9.38	17.83	−20.3
Molar 3rd	7–9 yr	12–16 yr	−9.49	16.53	−22.3	−8.09	18.01	−20.0
Bone			−11.67	12.89	−28.1			

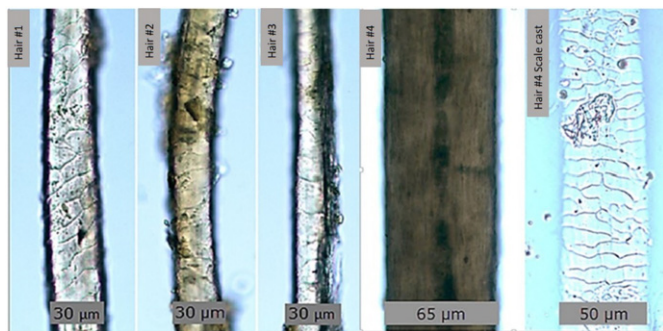


Fig. 6. Hairs that were adhered to and embedded within the pigment on the skull.

identified significant amounts (in mass %) of silicon, calcium, and iron (12.7%, 9.7%, and 3.3% respectively, Table 3). This suggests the pigment contains calcite, calcium silicate, iron silicate or iron oxide and is relatively raw, not a refined mineral like that used in artwork where much lower concentrations of impurities would be expected. Additional minor and trace elements were also identified (Table 1), notably Ni, which would be expected among other sulfides. XRF also suggested concentrations ≥ 1000 ppm of dysprosium, europium, and neodymium, but the more sensitive technique of LA-ICPMS did not support this, and we attribute the XRF results to peak overlaps from other elements such as iron and mercury. LA-ICPMS also identified low concentrations of lanthanum and cerium (Table 3). The goal of identifying these trace element concentrations and their specific combination within this cinnabar sample was to match it to a natural geological source. Although geochemical databases for cinnabar are as yet too sparse for application to this specimen, future work may help identify a region where the pigment originated.

4.6. mtDNA haplogroup

Initial quantification of the F112413 skull extracts and extraction blanks, as well as sequencing libraries with fluorometric quantification using the Qubit 2.0 assay indicated poor recovery of DNA from this individual. Fluorometric quantification measures the amount of total DNA in the extract but cannot indicate what percentage of the DNA is endogenous (belonging to the ancient individual). Ancient DNA extracts often contain contaminant DNA from other organisms such as bacteria, which are present in the burial environment or within the sample (Carpenter et al., 2013). DNA from these exogenous sources may be considered in the non-specific fluorometric measurement. For F112413, results from the quantification of all extracts indicated that the sample had very little DNA. Attempts to build complex sequencing libraries from these extracts were unsuccessful even after repeated amplification (Table 4).

To identify whether endogenous mtDNA was present in the extract and to check for contamination in the extraction blanks, we performed

Table 2

EDS elemental analysis for all pigment locations that contained mercury.

EDS spectra analysis for all pigment locations containing mercury (at%)					
Spectra location	N	O	S	Hg	Hg/S
SP003106	26.20	37.30	17.82	18.68	1.05
SP003108	21.59	17.48	28.79	32.14	1.12
SP003216		7.36	43.80	48.84	1.12
SP005118		28.80	36.33	34.87	0.96
SP005119	33.80	47.61	9.53	9.06	0.95
SP005120		52.24	21.04	26.72	1.27
SP005224		56.84	19.20	23.96	1.25
SP005225	48.42	24.75	13.43	13.40	1.00
SP005226		63.96	16.74	19.30	1.15
SP005227	52.27	18.12	13.11	16.50	1.26
SP005231			46.62	53.38	1.15
SP005533		19.85	42.62	37.53	0.88
SP005656		47.71	26.88	25.41	0.95
SP005659			51.55	48.45	0.94
SP005661		36.18	34.65	29.17	0.84
SP005662	37.18	43.38	10.50	8.94	0.85
Average	36.58	35.83	27.04	27.90	1.05

PCR targeting the non-coding region of human mtDNA located between the COII and tRNA^{lys} genes. This PCR is highly sensitive to small amounts of DNA, and it has the added benefit of testing for a marker that is characteristic of one of the four major Native American haplogroups (Merriwether et al., 1995; Schurr et al., 1990; Tamm et al., 2007; Wrischnik et al., 1987). The intergenic region between the cytochrome c oxidase subunit II (COII) gene and the tRNA gene for lysine typically contains two 9 bp repeats of the sequence CCCCTCTA. Deletion of one of those repeats is characteristic of mtDNA haplogroup B (Cann and Wilson, 1983; Wrischnik et al., 1987).

The PCR results suggest low amounts of DNA and occasional contamination of blanks or the sample itself. The latter was apparent because of the presence of two bands (i.e. one band with the 9 bp deletion and one larger band without the deletion). Specifically, our results suggest that there was some DNA in the F112413-1 and F112413-2 sample extracts. PCR results also suggested there was some DNA in the F112413 dental calculus (F112413-c), but there was a double band and the extraction blank was positive which indicates that contamination was introduced during the extraction process. Successful amplification of the 9 bp region was obtained with the F112413-3 extract. As illustrated in Fig. 8, skull F112413 carries the 9 bp deletion, indicating membership in haplogroup B.

5. Discussion

5.1. Skeletal analysis

Shape modification to skull F112413 presented particular difficulties of identifying affinity for repatriation in this case. There are no known

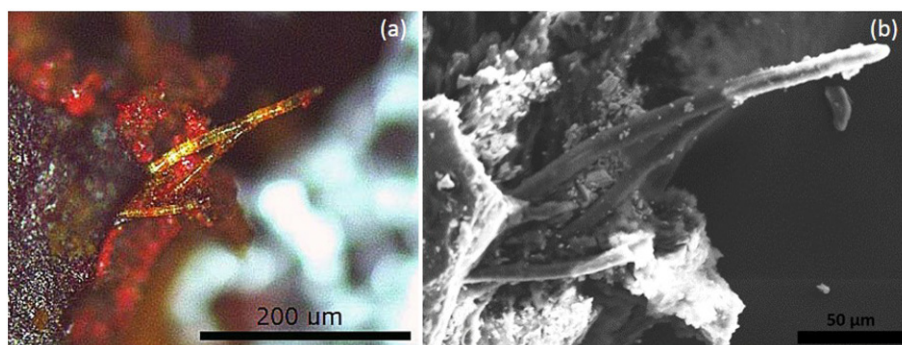


Fig. 7. (a) Red pigment, identified as cinnabar, with embedded hair under bright field optical microscopy at 5 \times magnification, and (b) the same sample under scanning electron microscopy.

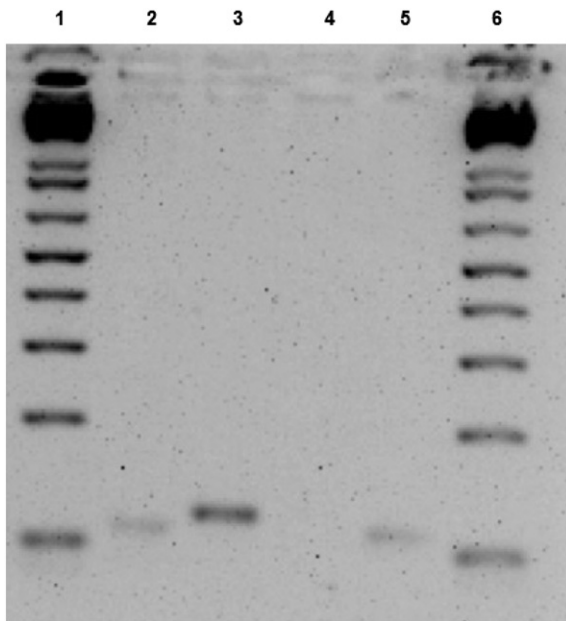


Fig. 8. Gel electrophoresis showing 9 bp deletion PCR. Lanes 1 and 6 show the DNA size standard. Lanes 2 and 5 show the results for F112413-3, lane 3 shows a positive control (without the deletion), and lane 4 shows the extraction blank (EB-3) from the F112413-3 extraction. The size of the band for F112413-3 is smaller than the band for the positive control, indicating the presence of the 9 bp deletion.

accounts of cranial deformation in Idaho prehistorically (Mark Plew, pers. comm., 2015), and there is no database of morphological or mortuary indicators to help identify remains to specific indigenous populations. Even if it were reported, cranial deformation alone is not a sufficiently specific cultural marker in North America to pinpoint the origins of these remains.

Ideally, a range of vault shapes and deformation characteristics from a population would be needed to accurately assess type and degree of vault modification and how such modification impacts sex and age estimations from the skull. If a skull shows signs of being deformed or otherwise intentionally altered, it is usually deemed unusable for such analyses using strict craniometrics analyses. More recently, authors have applied discriminant functions to identify presence/absence of deformation, and traditional and nonstandard craniometrics of deformed crania to establish indices of deformation type. Yet the impact of deformation on nonmetric traits remains unresolved (Cocilovo, 1975; Konigsberg et al., 1993; O'Brien and Stanley, 2013). Numerous facial and cranial base modifications may result from cranial vault deformation. Some authors (Cheverud et al., 1992) have determined that the number of morphological variations that ensue are too numerous,

Table 3

Trace elements present within the red pigment sample as identified via X-ray fluorescence (XRF) and laser ablation inductively coupled mass spectrometry (LA-ICPMS). Phosphorus had a sizeable contribution to the impurity concentration.

Trace elements identified via X-ray fluorescence				Trace elements identified via laser ablation inductively coupled mass spectrometry	
Element	Mass %	Element	ppm	Element	ppm
Silicon	12.7	Phosphorus	8300	Phosphorus	190
Calcium	9.7	Titanium	6000	Titanium	57
Iron	3.3	Zirconium	5400	Potassium	47
Potassium	2.2	Bromine	1400	Cerium	0.33
Aluminum	0.9	Nickel	1300	Neodymium	0.18
–	–	Manganese	1200	Lanthanum	0.09
–	–	–	–	Dysprosium	0.075
–	–	–	–	Europium	0.005

Table 4

Fluorometric quantification results for the F112413DNA extracts and libraries.

Sample	Stage	ng/uL
F112413-1	DNA extract (or dilution)	<0.50 ng/mL too low
EB-1		<0.50 ng/mL too low
F112413-1 1:10		1.46
EB-1 1:10		1.30
F112413-1 1:10 (again)	Indexed library	<0.50 ng/mL too low
EB-1 1:10 (again)		<0.50 ng/mL too low
F112413-1-A		2.96
EB-1-A		0.01
F112413-1-G	Amplified library	2.58
EB-1-G		0.01
LB		0.01
F112413-1-A		189.00
EB-1-A		11.50
F112413-1-G		36.00
EB-1-G		22.20
LB		14.60

dramatic, and global to allow for analysis of population affinity and relationships in deformed crania from nonmetric traits, while others (Kohn et al., 1995; Konigsberg et al., 1993) have found the effects to be minimal and local (limited to a few ossicles and foramina) and not barriers to interpretation of biological distance. This means that when unprovenanced skeletal remains are discovered in the state of Idaho, and likely in other locations, it is an ongoing challenge to ethically curate even after a Notice of Intent to Repatriate has been given.

Overall, cranial features, combined with what is known about the specimen history/origins, cranial modification, and the adhering pigment and hairs, are consistent with a Native American ancestry for F112413. That being said, given the unknown context of the remains and the fact that neither the cranial morphology or deformation, nor the pigment, are able to specifically assign origin, this ancestry estimation from the cranium was not considered informed enough to proceed with repatriation alone. With a lack of archaeological background, these above data were not enough to identify the origins as being necessarily North American, let alone from Idaho, in order to repatriate, and spurred further investigatory methods.

5.2. Stable isotopes

Because $\delta^{13}\text{C}$ values of C3 plants increase in arid ecosystems, such as in Idaho, C3 plants likely formed the majority of this individual's diet, but some consumption of high $\delta^{13}\text{C}$ foods is possible. C4 type plants have never been a large part of the resource base in the Pacific Northwest (Kohn and Law, 2006). Migrating Pacific salmon have elevated $\delta^{13}\text{C}$ values, and impart their unique isotopic signature to their spawning freshwater environment (Bilby et al., 1996). Consumption of salmon or foods in salmon-bearing freshwater environments could have helped elevate tooth $\delta^{13}\text{C}$ of the individual we analyzed.

Before interpreting $\delta^{18}\text{O}$ values, we argue that isotope compositions at c. 1300–1400 CE should not have been markedly different from today, so modern isotope patterns can be used for interpretations. After a global maximum temperature at c. 1000 CE during the Medieval Warm Period, and prior to modern climate change, northern hemisphere temperatures gradually declined to a minimum during the Little Ice Age (c. 1600–1700 CE; Mann et al., 1999; Stocker et al., 2014). The age of F112413 coincides with an intermediate position between maximum and minimum temperatures such that it approximates modern conditions. For example, the detailed record of Dahl-Jensen et al. (1998) for the Greenland ice core indicates that temperatures at 1300–1400 CE are not resolvably different from modern day. Insofar as isotope compositions generally track temperatures, we would not expect isotope compositions at 1300–1400 CE to be significantly different from today. Several studies corroborate this expectation in the northwestern US. Proxy records include speleothems from southeastern Idaho (Lundeen

et al., 2013) and near-coastal Oregon (Ersek et al., 2009), as well as lake sediments from the northern interior Great Basin (Benson et al., 2002), and extreme SE Idaho (Dean et al., 2005). Lundeen et al. (2013) interpret their data in terms of temperature (assuming no change to $\delta^{18}\text{O}$ of precipitation), and the other records suggest no shift to $\delta^{18}\text{O}$. Depending on assumptions regarding temperature shifts, data from Lundeen et al. (2013) might be reconciled with a maximum decrease in $\delta^{18}\text{O}$ for 1300–1400 CE relative to today of -1% . Thus we take into account possible downward shift of 0–1%.

The inferred $\delta^{18}\text{O}_{\text{IW}}$ values (-20 to -22% from enamel; -28% from bone) are extremely low and point to either reduced temperatures at high latitude or high altitude (Dansgaard, 1964). The highest passes in central Idaho (2200 to 2800 m) have snowpack $\delta^{18}\text{O}$ values of -18 to -20% (Anderson et al., 2016), or somewhat too high to explain the tooth enamel compositions, even assuming a 1‰ increase in regional $\delta^{18}\text{O}$ since 1300–1400 CE, and far too high to explain bone compositions. There is no evidence of sustained residential occupation at altitudes >2000 m in Idaho (Bishop and Plew, 2016; Morgan et al., 2012; Murphy and Murphy, 1960), and ingested water is also likely to be acquired from streams, which have higher $\delta^{18}\text{O}_{\text{IW}}$ values than snowpack. Instead, the $\delta^{18}\text{O}_{\text{IW}}$ values of the tooth and bone suggest F112413 consumed water that was derived from an area with a sustained reduced average temperature compared to modern and prehistoric Idaho values. Enamel $\delta^{18}\text{O}_{\text{IW}}$ values can be found for modern surface water in Canada and Alaska, suggesting the range of locations possible during this individual's early years (Fig. 5). Bone isotope values appear to reflect migration to an even higher latitude or perhaps to the Canadian Rockies.

5.3. Hair and pigment

Mercury sulfide is a red pigment that has been used in funerary and other symbolic rituals dating back to the Neolithic period (Ávila et al., 2014; Rogerio-Candelera et al., 2013). Red colored pigments are documented to have been used throughout western North America for body painting on festive or mourning occasions, for utilitarian and symbolic decoration of materials, and for skin protection and healing (Heizer and Treganya, 1944). It is interesting to note that there may have been knowledge of the ill effects of the mercury in cinnabar, as a recounting by Chief Umunhun warned warriors to wash themselves of the pigment to cure themselves of illness (Heizer and Treganya, 1944).

Historically, several surface outcroppings of cinnabar (also commonly known as vermilion when referred to as a pigment) near San Jose and Sacramento, California were said to be well known among Native Americans from northern California to the Columbia River in the early 1800s (and most likely longer before this). Heizer and Fenenga (1939) believed it probable that the 6 lb of cinnabar recovered from historic Native American burials in Contra Costa County, California originated from these mining locations. The Northwestern Chinook tribe was also known to have traded in cinnabar which they obtained from other native tribes in southern Oregon (Swan, 1870). A typical Chinook burial involved placing the deceased in a cabin or raised canoe. Bodies were reportedly painted with ochre and then wrapped in several mats, hides, or blankets (Ruby and Brown, 1976). It is possible that this “ochre” may have in fact been cinnabar. The animal hairs found adhering to the skull F112413 then, are probable remnants of funerary shrouds covering a cinnabar painted body. Since all hairs were found adhering to the skull because they were embedded in the pigment, it is likely the cinnabar was applied before soft tissue decomposition. One famous Chinook leader, Chief Comcomly, who died in 1830, was buried in this traditional manner; his skull was later removed and his body was buried along his lands (Ruby and Brown, 1976). Removal of the skull from the body prior to burial could explain why the skull of F112413 was the only skeletal component retrieved in Idaho.

5.4. mtDNA

As previously stated, the PCR results from the F112413-3 sample showed the 9 bp deletion indicative of membership in mtDNA Haplogroup B. This haplogroup is common in the Americas but has also been identified in Polynesia. However, Polynesian lineages are distinct from Native American lineages (Melton et al., 1995). In addition, the 9 bp deletion arose independently in Africa where it is found on a different haplogroup background (Soodyall et al., 1996). Discerning the specific haplotype would require sequencing of at least a portion of the mtDNA genome which is not possible given the level of DNA preservation of F112413.

6. Conclusions

Murad and Murad (2000) were successful in reuniting a disinterred Native American skull with the rest of the body from a California cemetery, and Seidemann et al. (2009) were able to determine the likely burial location of a Native American skull listed for sale on eBay. Unfortunately, such a degree of success is not always possible for unprovenienced remains. The bioarchaeological record of Idaho is not well documented, primarily due to avoidance of burial excavations or speedy repatriation. These practices allow for preservation of invaluable remains and maintenance of the relationship among bioarchaeologists and indigenous descent populations. However, they result in limited reference collections and data from which to compare skeletal remains similar to F112413 which require of affiliation to carry out NAGPRA. However, the results of this multidisciplinary study for determining the likely origins and affinity of the skull provided much more insight about F112413 than any one direction of inquiry. Bioarchaeological assessments identified intentional cranial deformation and a post-or ante-mortem application of a red pigment. Analysis of the hair embedded within the pigment identified two types: human hair and that of an animal, possibly sheep, cattle, deer, dog, marmot or musk ox. Although there was a limited provenance provided for the F112413, these initial findings provided the basis for the hypothesis that the skull was of Native American origin. The pigment was identified as cinnabar, or mercury sulfide, and trace element concentrations were detected in amounts which suggest the pigment was of a natural origin and was not a refined colorant. The Pacific Northwestern Chinook tribe was known to have practiced cranial deformation and were traders of vermilion, or cinnabar, a brilliant red pigment. It has also been documented that they practiced post-mortem application of red pigments on their deceased as well as on other funerary items. Stable isotope analysis confirmed that the habitat in which the individual lived was either cooler in the years closer to the person's death or the individual lived in a different location in which the climate was cooler and/or at a higher altitude. Comparisons from the teeth and bone from the skull do not conform to expectations for isotopic data in the Idaho region, however, other areas in the Pacific Northwest and up into Alaska, where the Chinook were known to have trade routes, could be potential matches, rather than Idaho. Radiocarbon dating was performed on a bone sample verifying the skull is prehistoric. Mitochondrial DNA analysis revealed a 9 bp deletion found frequently in haplogroup B lineages of Native Americans. More work would be needed to discern the specific haplotype, although this is improbable given poor DNA preservation.

The inability to more specifically identify a cultural group to which the F112413 skull may be affiliated underscores the need for more extensive comparative collections in osteology and the necessity for trace element analysis of archaeologically used minerals like cinnabar (Seidemann et al., 2009). Despite that, this case demonstrates the potential of combining multiple analyses for narrowing the geographic identification of unprovenienced remains. The F112413 skull was returned to the State Historic Preservation Office of Idaho and is awaiting repatriation. Even though the skull in this case could not be reunited with the rest of the body or linked to an exact origin, it is

hoped that the ability to now return the remains to a geographically appropriate cultural descendant group will provide some reassurance to Northwestern indigenous communities that the State of Idaho takes NAGPRA and other indigenous concerns seriously.

Acknowledgements

We are grateful the Ken Reid and the State Historic Preservation Office for loaning the skeletal remains to BSU for analysis and for his repatriation efforts. We acknowledge Vicki Cassman and Elizabeth Shaeffer at the University of Delaware for their assistance with the hair analysis. Thank you also to Tammy Buonasera (University of Arizona) and Mark Plew (BSU) for firsthand background of cinnabar and Idaho archaeology. We thank Samantha Evans and Robin Trayler, BSU Geoscience, who helped guide tooth and bone processing and collected stable isotope data. We thank Darden Hood at Beta Analytic for his contributions and expertise in radiocarbon dating methods and theory. We also thank doctoral student Tanvi Honap and post-doctoral researcher Andrew Ozga with Arizona State University for their contributions to the DNA analysis. This research was supported through NSF Grants EAR0842367 and EAR0819837.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.jasrep.2017.02.006>.

References

- Anderson, L., Berkelhammer, M., Mast, M.A., 2016. Isotopes in North American Rocky Mountain Snowpack 1993–2014. *Quat. Sci. Rev.* 131:262–273. <http://dx.doi.org/10.1016/j.quascirev.2015.03.023>.
- Antón, S.C., 1989. Intentional cranial vault deformation and induced changes of the cranial base and face. *Am. J. Phys. Anthropol.* 79:253–267. <http://dx.doi.org/10.1002/ajpa.1330790213>.
- Antón, S.C., Weinstein, K.J., 1999. Artificial cranial deformation and fossil Australians revisited. *J. Hum. Evol.* 36:195–209. <http://dx.doi.org/10.1006/jhev.1998.0266>.
- Appleyard, H.M., 1978. *Guide to the Identification of Animal Fibres*.
- Ávila, A., Mansilla, J., Bosch, P., Pijoan, C., 2014. Cinnabar in Mesoamerica: poisoning or mortuary ritual? *J. Archaeol. Sci.* 49:48–56. <http://dx.doi.org/10.1016/j.jas.2014.04.024>.
- Bauska, T.K., Joos, F., Mix, A.C., Roth, R., Ahn, J., Brook, E.J., 2015. Links between atmospheric carbon dioxide, the land carbon reservoir and climate over the past millennium. *Nat. Geosci.* 8, 383–387.
- Benson, L., Kashgarian, M., Rye, R., Lund, S., Paillet, F., Smoot, J., Kester, C., Mensing, S., Meko, D., Lindström, S., 2002. Holocene multidecadal and multicentennial droughts affecting Northern California and Nevada. *Quat. Sci. Rev.* 21, 659–682.
- Bilby, R.E., Franssen, B.R., Bisson, P.A., 1996. Incorporation of nitrogen and carbon from spawning coho salmon into the trophic system of small streams: evidence from stable isotopes. *Can. J. Fish. Aquat. Sci.* 53:164–173. <http://dx.doi.org/10.1139/f95-159>.
- Bishop, M., Plew, M.G., 2016. Fuel exploitation as a factor in Shoshone winter mobility. *North Am. Archaeol.* 37:3–19. <http://dx.doi.org/10.1177/0197693115594505>.
- Boas, F., 1890. *Second general report on the Indians of British Columbia*. Br. Assoc. Adv. Sci. London 562–715.
- Brown, P., 1981. Artificial cranial deformation: a component in the variation in Pleistocene Australian Aboriginal crania. *Archaeol. Ocean.* 16, 156–167.
- Buikstra, J.E., Ubelaker, D.H., 1994. *Standards for Data Collection From Human Skeletal Remains*.
- Cann, R.L., Wilson, A.C., 1983. Length mutations in human mitochondrial-DNA. *Genetics* 104, 699–711.
- Carpenter, M.L., Buenrostro, J.D., Valdiesera, C., Schroeder, H., Allentoft, M.E., Sikora, M., Rasmussen, M., Gravel, S., Guillén, S., Nekhrizov, G., 2013. Pulling out the 1%: whole-genome capture for the targeted enrichment of ancient DNA sequencing libraries. *Am. J. Hum. Genet.* 93, 852–864.
- Cerling, T.E., Harris, J.M., MacFadden, B.J., Leakey, M.G., Quade, J., Eisenmann, V., Ehleringer, J.R., 1997. Global vegetation change through the Miocene/Pliocene boundary. *Nature* 389, 153–158.
- Cheney, C.A., Pashley, V., Lamb, A.L., Sloane, H.J., Evans, J.A., 2012. The oxygen isotope relationship between the phosphate and structural carbonate fractions of human bioapatite. *Rapid Commun. Mass Spectrom.* 25, 309–319.
- Cheverud, J.M., Kohn, L.A.P., Konigsberg, L.W., Leigh, S.R., 1992. Effects of fronto-occipital artificial cranial vault modification on the cranial base and face. *Am. J. Phys. Anthropol.* 88:323–345. <http://dx.doi.org/10.1002/ajpa.1330880307>.
- Clark, J.L., Dobson, S.D., Antón, S.C., Hawks, J., Hunley, K.L., Wolpoff, M.H., 2007. Identifying artificially deformed crania. *Int. J. Osteoarchaeol.* 17:596–607. <http://dx.doi.org/10.1002/oa.910>.
- Clementz, M.T., Fox-Dobbs, K., Wheatley, P.V., Koch, P.L., Doak, D.F., 2009. Revisiting old bones: coupled carbon isotope analysis of bioapatite and collagen as an ecological and palaeoecological tool. *Geol. J.* 44:605–620. <http://dx.doi.org/10.1002/gj.1173>.
- Cocilovo, J.A., 1975. Estudio de dos factores que influyen la morfología craneana en una colección andina: el sexo y la deformación artificial. *Rev. del Inst. Antropol.* 3, 197–212.
- Dabney, J., Knapp, M., Glocke, I., Gansauge, M.T., Weihmann, A., Nickel, B., Valdiosera, C., Garcia, N., Paabo, S., Arsuaga, J.L., Meyer, M., 2013. Complete mitochondrial genome sequence of a Middle Pleistocene cave bear reconstructed from ultrashort DNA fragments. *Proc. Natl. Acad. Sci. U. S. A.* 110:15758–15763. <http://dx.doi.org/10.1073/pnas.1314445110>.
- Dahl-Jensen, D., Mosegaard, K., Gundestrup, N., Clow, G.D., Johnsen, S.J., Hansen, A.W., Balling, N., 1998. Past temperatures directly from the Greenland ice sheet. *Science* 282 (80), 268–271.
- Dansgaard, W., 1964. *Stable isotopes in precipitation*. Tellus 16, 436–468.
- Daux, V., Lecuyer, C., Heran, M.A., Amiot, R., Simon, L., Fourel, F., Martineau, F., Lynnerup, N., Reychler, H., Escarguel, G., 2008. Oxygen isotope fractionation between human phosphate and water revisited. *J. Hum. Evol.* 55 (6):1138–1147. <http://dx.doi.org/10.1016/j.jhev.2008.06.006>.
- Dean, W., Forester, R., Colman, S., Liu, A., Skipp, G., Simmons, K., Swarzenski, P., Anderson, R., 2005. *Modern and Glacial-Holocene Carbonate Sedimentation in Bear Lake, Utah-Idaho*.
- Dingwall, E.J., 1931. *Artificial Cranial Deformation; a Contribution to the Study of Ethnic Mutilations*. Bale, London.
- Edgar, H.J.H., 2013. Estimation of ancestry using dental morphological characteristics. *J. Forensic Sci.* 58:S3–S8. <http://dx.doi.org/10.1111/j.1556-4029.2012.02295.x>.
- Erlandson, J.M., Robertson, J.D., Descantes, C., 1999. Geochemical analysis of eight red ochres from western North America. *Am. Antiq.* 64:517–526. <http://dx.doi.org/10.2307/2694149>.
- Ersek, V., Hostetler, S.W., Cheng, H., Clark, P.U., Anslow, F.S., Mix, A.C., Edwards, R.L., 2009. Environmental influences on speleothem growth in southwestern Oregon during the last 380,000 years. *Earth Planet. Sci. Lett.* 279, 316–325.
- France, D.L., 1998. Observational and metric analysis of sex in the skeleton. *Forensic Osteol. Adv. Identif. Hum. Remains*. Charles C Thomas, Springfield, 163–186.
- Friedli, H., Löffler, H., Oeschger, H., Siegenthaler, U., Stauffer, B., 1986. Ice core record of the C-13/C-12 ratio of atmospheric CO₂ in the past two centuries. *Nature* 324, 237.
- Gerszten, P.C., 1993. An investigation into the practice of cranial deformation among the Pre-Columbian peoples of northern Chile. *OA Int. J. Osteoarchaeol.* 3, 87–98.
- Gilbert, M.T.P., Hansen, A.J., Willerslev, E., Turner-Walker, G., Collins, M., 2006. Insights into the processes behind the contamination of degraded human teeth and bone samples with exogenous sources of DNA. *Int. J. Osteoarchaeol.* 16:156–164. <http://dx.doi.org/10.1002/oa.832>.
- Giles, R.E., Blanc, H., Cann, H.M., Wallace, D.C., 1980. Maternal inheritance of human mitochondrial DNA. *Proc. Natl. Acad. Sci.* 77, 6715–6719.
- Good, S.P., Noone, D., Bowen, G., 2015. Hydrologic connectivity constrains partitioning of global terrestrial water fluxes. *Science* 349 (80), 175–177.
- Gruspier, K., Mullen, G., 1991. Maxillary suture obliteration: a test of the Mann method. *J. Forensic Sci.* 36, 512–519.
- Heizer, R.F., Fenenga, F., 1939. *Archaeological horizons in central California*. Am. Anthropol. 41, 378–399.
- Heizer, R., Treganya, A., 1944. *California Indian mines and quarries*. Calif. J. Mines Geol. 40, 291–359.
- Hu, S.S., Fernandez, S.A., Hill, K.J., Li, Y., 2006. Remodeling dynamics in the alveolar process in skeletally mature dogs. *Anat. Rec. A Discov. Mol. Cell. Evol. Biol.* 288, 1243–1249.
- Kendall, C., Coplen, T.B., 2001. Distribution of oxygen-18 and deuterium in river waters across the United States. *Hydrol. Process.* 15:1363–1393. <http://dx.doi.org/10.1002/hyp.217>.
- Koch, P.L., 1998. Isotopic reconstruction of past continental environments. *Annu. Rev. Earth Planet. Sci.* 26:573–613. <http://dx.doi.org/10.1146/annurev.earth.26.1.573>.
- Koch, P.L., Tuross, N., Fogel, M.L., 1997. The effects of sample treatment and diagenesis on the isotopic integrity of carbonate in biogenic hydroxylapatite. *J. Archaeol. Sci.* 24: 417–429. <http://dx.doi.org/10.1006/jasc.1996.0126>.
- Kohn, M.J., 2010. Carbon isotope compositions of terrestrial C3 plants as indicators of (paleo)ecology and (paleo)climate. *Proc. Natl. Acad. Sci. U. S. A.* 107:19691–19695. <http://dx.doi.org/10.1073/pnas.1004933107>.
- Kohn, M.J., Cerling, T.E., 2002. Stable isotope compositions of biological apatite. *Phosphates Geochim. Geobiol. Mater. Importance.* 48:pp. 455–488. <http://dx.doi.org/10.2138/rmg.2002.48.12>.
- Kohn, M.J., Law, J.M., 2006. Stable isotope chemistry of fossil bone as a new paleoclimate indicator. *Geochim. Cosmochim. Acta* 70:931–946. <http://dx.doi.org/10.1016/j.gca.2005.10.023>.
- Kohn, L.A.P., Leigh, S.R., Cheverud, J.M., 1995. Asymmetric vault modification in Hopi crania. *Am. J. Phys. Anthropol.* 98, 173–195.
- Kohn, M.J., Strömberg, C.A.E., Madden, R.H., Dunn, R.E., Evans, S., Palacios, A., Carlini, A.A., 2015. Quasi-static Eocene–Oligocene climate in Patagonia promotes slow faunal evolution and mid-Cenozoic global cooling. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 435, 24–37.
- Konigsberg, L.W., Kohn, L.A.P., Cheverud, J.M., 1993. Cranial deformation and nonmetric trait variation. *Am. J. Phys. Anthropol.* 90:35–48. <http://dx.doi.org/10.1002/ajpa.1330900103>.
- Krogman, W.M., 1962. *The Human Skeleton in Forensic Medicine*. Thomas, Springfield, Ill.
- Lundeen, Z., Brunelle, A., Burns, S.J., Polyak, V., Asmerom, Y., 2013. A speleothem record of Holocene paleoclimate from the northern Wasatch Mountains, southeast Idaho, USA. *Quat. Int.* 310, 83–95.
- Luz, B., Kolodny, Y., Horowitz, M., 1984. Fractionation of oxygen isotopes between mammalian bone phosphate and environmental drinking water. *Geochim. Cosmochim. Acta* 48:1689–1693. [http://dx.doi.org/10.1016/0016-7037\(84\)90338-7](http://dx.doi.org/10.1016/0016-7037(84)90338-7).

- Mann, R.W., Symes, S.A., Bass, W.M., 1987. Maxillary suture obliteration – aging the human skeleton based on intact or fragmentary maxilla. *J. Forensic Sci.* 32, 148–157.
- Mann, M.E., Bradley, R.S., Hughes, M.K., 1999. Northern hemisphere temperatures during the past millennium: inferences, uncertainties, and limitations. *Geophys. Res. Lett.* 26, 759–762.
- Meindl, R.S., Lovejoy, C.O., 1985. Ectocranial suture closure: a revised method for the determination of skeletal age at death based on the lateral-anterior sutures. *Am. J. Phys. Anthropol.* 68, 57–66.
- Melton, T., Peterson, R., Redd, A.J., Saha, N., Sofro, A.S.M., Martinson, J., Stoneking, M., 1995. Polynesian genetic affinities with Southeast Asian populations as identified by mtDNA analysis. *Am. J. Hum. Genet.* 57, 403–414.
- Merriwether, D.A., Rothhammer, F., Ferrell, R.E., 1995. Distribution of the four founding lineage haplotypes in native Americans suggests a single wave of migration for the New World. *Am. J. Phys. Anthropol.* 98:411–430. <http://dx.doi.org/10.1002/ajpa.1330980404>.
- Minganti, V., Capelli, R., Drava, G., De Pellegrini, R., 2007. Solubilization and methylation of HgS, PbS, and SnS by iodomethane, a model experiment for the aquatic environment. *Chemosphere* 67:1018–1024. <http://dx.doi.org/10.1016/j.chemosphere.2006.10.053>.
- Morgan, C., Losey, A., Adams, R., 2012. High-altitude hunter-gatherer residential occupations in Wyoming's Wind River Range. *North Am. Archaeol.* 33:35–79. <http://dx.doi.org/10.2190/NA.33.1.d>.
- Murad, T., Murad, T., 2000. The postmortem fate of Pat Gregory: a disinterred Native American. *J. Forensic Sci.* 45, 488–494.
- Murphy, R.F., Murphy, Y., 1960. Shoshone-Bannock Subsistence and Society. *Anthropological Records* No. 9. Univ. California, Berkeley, pp. 235–273.
- O'Brien, T.G., Stanley, A.M., 2013. Boards and cords: discriminating types of artificial cranial deformation in prehispanic South Central Andean populations. *Int. J. Osteoarchaeol.* 23:459–470. <http://dx.doi.org/10.1002/oa.1269>.
- O'Loughlin, V.D., 2004. Effects of different kinds of cranial deformation on the incidence of wormian bones. *Am. J. Phys. Anthropol.* 123:146–155. <http://dx.doi.org/10.1002/ajpa.10304>.
- Ortner, D.J., 2003. *Identification of Pathological Conditions in Human Skeletal Remains*. Academic Press.
- Pääbo, S., 1989. Ancient DNA: extraction, characterization, molecular-cloning, and enzymatic amplification. *Proc. Natl. Acad. Sci. U. S. A.* 86:1939–1943. <http://dx.doi.org/10.1073/pnas.86.6.1939>.
- Pasteris, J.D., Wopenka, B., Freeman, J.J., Rogers, K., Valsami-Jones, E., van der Houwen, J.A.M., Silva, M.J., 2004. Lack of OH in nanocrystalline apatite as a function of degree of atomic order: implications for bone and biomaterials. *Biomaterials* 25:229–238. [http://dx.doi.org/10.1016/S0142-9612\(03\)00487-3](http://dx.doi.org/10.1016/S0142-9612(03)00487-3).
- Prowse, T.L., Schwarcz, H.R., Garnsey, P., Knyf, M., Macchiarelli, R., Bondioli, L., 2007. Isotopic evidence for age-related immigration to imperial Rome. *Am. J. Phys. Anthropol.* 132:510–519. <http://dx.doi.org/10.1002/ajpa.20541>.
- Reimer, P.J., Bard, E., Bayliss, A., Beck, J.W., Blackwell, P.G., Ramsey, C.B., Buck, C.E., Cheng, H., Edwards, R.L., Friedrich, M., 2013. *IntCal13 and Marine13 radiocarbon age calibration curves 0–50,000 years cal BP*. *Radiocarbon* 55, 1869–1887.
- Rhine, S., 1990. Non-metric skull racing. *Skelet. Attrib. Race* 9–20.
- Rogério-Candelera, M.Á., Herrera, L.K., Miller, A.Z., García Sanjuán, L., Mora Molina, C., Wheatley, D.W., Justo, Á., Saiz-Jimenez, C., 2013. Allochthonous red pigments used in burial practices at the Copper Age site of Valencina de la Concepción (Sevilla, Spain): characterisation and social dimension. *J. Archaeol. Sci.* 40:279–290. <http://dx.doi.org/10.1016/j.jas.2012.08.004>.
- Rohland, N., Hofreiter, M., 2007. Ancient DNA extraction from bones and teeth. *Nat. Protoc.* 2:1756–1762. <http://dx.doi.org/10.1038/nprot.2007.247>.
- Ruby, R.H., Brown, J.A., 1976. *The Chinook Indians: Traders of the Lower Columbia River*. University of Oklahoma Press, Norman.
- Sandberg, P.A., Loudon, J.E., Sponheimer, M., 2012. Stable isotope analysis in primatology: a critical review. *Am. J. Primatol.* 74:969–989. <http://dx.doi.org/10.1002/ajp.22053>.
- Satterfield, F.R., Finney, B.P., 2002. Stable isotope analysis of Pacific salmon: insight into trophic status and oceanographic conditions over the last 30 years. *Prog. Oceanogr.* 53, 231–246.
- Schoeninger, M.J., DeNiro, M.J., 1984. Nitrogen and carbon isotopic composition of bone collagen from marine and terrestrial animals. *Geochim. Cosmochim. Acta* 48, 625–639.
- Schuenemann, V.J., Bos, K., DeWitte, S., Schmedes, S., Jamieson, J., Mittnik, A., Forrest, S., Coombes, B.K., Wood, J.W., Earn, D.J.D., White, W., Krause, J., Poinar, H.N., 2011. Targeted enrichment of ancient pathogens yielding the pPCP1 plasmid of *Yersinia pestis* from victims of the Black Death. *Proc. Natl. Acad. Sci. U. S. A.* 108:E746–E752. <http://dx.doi.org/10.1073/pnas.1105107108>.
- Schurr, T.G., Ballinger, S.W., Gan, Y.Y., Hodge, J.A., Merriwether, D.A., Lawrence, D.N., Knowler, W.C., Weiss, K.M., Wallace, D.C., 1990. Amerindian mitochondrial DNAs have rare Asian mutations at high frequencies, suggesting they derived from four primary maternal lineages. *Am. J. Hum. Genet.* 46, 613–623.
- Seidemann, R.M., Stojanowski, C.M., Rich, F.J., 2009. The identification of a human skull recovered from an eBay sale. *J. Forensic Sci.* 54:1247–1253. <http://dx.doi.org/10.1111/j.1556-4029.2009.01194.x>.
- Soodyall, H., Vigilant, L., Hill, A.V., Stoneking, M., Jenkins, T., 1996. mtDNA control-region sequence variation suggests multiple independent origins of an "Asian-specific" 9-bp deletion in sub-Saharan Africans. *Am. J. Hum. Genet.* 58, 595–608.
- Stocker, T., Change, I.P. on C, Change, I.P. on C, I., W.G., 2014. *Climate Change 2013: The Physical Science Basis: Working Group I Contribution to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*.
- Swan, J.G., 1870. *The Indians of Cape Flattery, at the Entrance to the Strait of Fuca, Washington Territory*. Collins, Philadelphia.
- Tamm, E., 2007. Beringian Standstill and Spread of Native American Founders. *PLoS One* vol. 2 (no. 9), e829 (Sep.).
- Walker, P.L., 2008. Sexing skulls using discriminant function analysis of visually assessed traits. *Am. J. Phys. Anthropol.* 136:39–50. <http://dx.doi.org/10.1002/ajpa.20776>.
- Warinner, C., Rodrigues, J.F.M., Vyas, R., Trachsel, C., Shved, N., Grossmann, J., Radini, A., Hancock, Y., Tito, R.Y., Fiddymment, S., Speller, C., Hendy, J., Charlton, S., Luder, H.U., Salazar-Garcia, D.C., Eppler, E., Seiler, R., Hansen, L.H., Castruita, J.A.S., Barkow-Oesterreicher, S., Teoh, K.Y., Kelstrup, C.D., Olsen, J.V., Nanni, P., Kawai, T., Willerslev, E., von Mering, C., Lewis, C.M., Collins, M.J., Gilbert, M.T.P., Rühli, F., Cappellini, E., 2014. Pathogens and host immunity in the ancient human oral cavity. *Nat. Genet.* 46 (336–+). <http://dx.doi.org/10.1038/ng.2906>.
- Wreschner, E.E., Bolton, R., Butzer, K.W., Delporte, H., Häusler, A., Heinrich, A., Jacobson-Widding, A., Malinowski, T., Masset, C., Miller, S.F., Ronen, A., Solecki, R., Stephenson, P.H., Thomas, L.L., Zollinger, H., 1980. Red ochre and human evolution: a case for discussion [and comments and reply]. *Curr. Anthropol.* 21:631–644. <http://dx.doi.org/10.2307/2741829>.
- Wrischnik, L.A., Higuchi, R.G., Stoneking, M., Erlich, H.A., Arnheim, N., Wilson, A.C., 1987. Length mutations in human mitochondrial DNA: direct sequencing of enzymatically amplified DNA. *Nucleic Acids Res.* 15:529–541. <http://dx.doi.org/10.1093/nar/15.2.529>.