

# Working Ethically with Ancient DNA from Composites in the United States

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## ABSTRACT

This article discusses ethical frameworks for planning and implementing composite research in the United States. Composites, defined here as archaeological materials with multiple genetic sources, include materials such as sediment, coprolites, birch pitch, and dental calculus. Although composites are increasingly used in genetic research, the ethical considerations of their use in ancient DNA studies have not been widely discussed. Here, we consider how composites' compositions, contexts, and potential to act as proxies can affect research plans and offer an overview of the primary ethical concerns of ancient DNA research. It is our view that ethical principles established for analyses of Ancestral remains and related materials can be used to inform research plans when working with composite evidence. This work also provides a guide to archaeologists unfamiliar with genetics analyses in planning research when using composite evidence from the United States with a focus on collaboration, having a clear research plan, and using lab methods that provide the desired data with minimal destruction. Following the principles discussed in this article and others allows for engaging in composite research while creating and maintaining positive relationships with stakeholders.

**Keywords:** Ancient DNA, genetics, research ethics, collaborative archaeology, coprolites, sediment, dental calculus, birch pitch

El presente trabajo analiza las preocupaciones éticas para la planificación e implementación de investigaciones compuestas en los Estados Unidos. Los compuestos, definidos aquí como muestras arqueológicas con múltiples fuentes genéticas, incluyen materiales como sedimentos, paleofecas, brea de abedul y calculo dental. Sin embargo, si bien los compuestos se han utilizado cada vez más en la investigación genética arqueológica, las consideraciones éticas de su uso en estudios de aADN no se han discutido ampliamente. Aquí consideramos cómo las composiciones, los contextos y el potencial de los compuestos para actuar como sustitutos pueden afectar los planes de investigación y ofrecer una visión general de las principales preocupaciones éticas de la investigación del ADN antiguo. Es la opinión de los autores que los principios éticos establecidos para los análisis de restos humanos y materiales relacionados se pueden utilizar para informar los planes de investigación cuando se trabaja con evidencia compuesta. Este trabajo ofrece también una guía para planificar la investigación cuando se utiliza evidencia compuesta con un enfoque en la colaboración, en planes de investigación claros y uso de métodos de laboratorio que proporcionen los datos deseados con una destrucción mínima de la muestra. Seguir los principios descritos en este documento permite participar en la investigación compuesta sin dejar de lado la creación y mantención de relaciones positivas con las partes interesadas.

**Palabras clave:** ADN antiguo, genética, ética de la investigación, arqueología colaborativa, paleofecas, sedimento, calculo dental, alquitrán de abedul

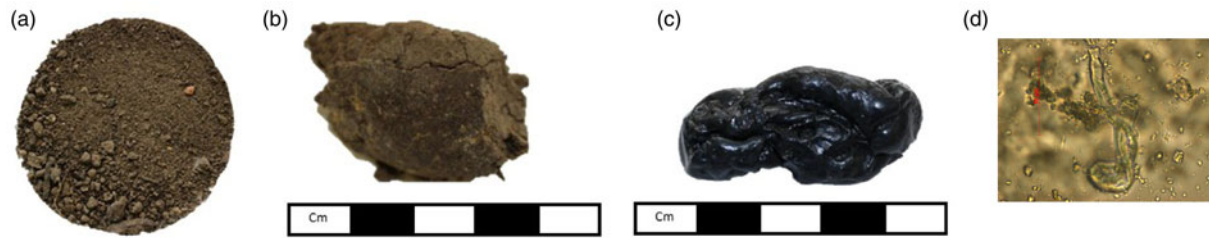
Ancient DNA (aDNA) has increasingly been used to answer and elucidate many questions about human origins and existence, capturing the interest of both the scientific community and the public. It has expanded the possibility of studying the genetic pasts of human Ancestors, the plants and animals humans interacted with, the microbes that inhabited them, and the environments that influenced their development. The bulk of paleogenomic work has been performed on Ancestor remains (Brunson and Reich 2019; Liu et al. 2021), but environmental and nonhuman materials have increasingly been used (Armbrecht et al. 2019; Crump 2021; Shillito et al. 2020). One category of such materials is composites, or materials containing DNA from multiple organisms that include flora, fauna,

microbes, and humans. Composites may also contain a variety of archaeological materials—such as macro, micro, and additional molecular remains—making composite research inherently multiproxy. The composites discussed here are sediment, coprolites, birch pitch, and dental calculus (Figure 1). A single composite could contain plant and animal remains, pollen, phytoliths, diatoms, parasites, proteins, lipid biomarkers, and DNA, each of which could be the subject of analysis. Composites can further act as proxies for more sensitive materials, but their use in research is not free from the ethical concerns raised by stakeholders and researchers who are engaged in, associated with, or affected by genetic research.

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**FIGURE 1.** Composites discussed in this article: (a) sediment, (b) coprolite, (c) pine pitch, and (d) dental calculus under a microscope. As shown, coprolite and pine pitch are morphologically unique, whereas sediment is not; different amounts of documentation are needed. All composites may also contain a mixture of visible and molecular remains. (Sediment, coprolite, and pitch photos provided by Taryn Johnson. Dental calculus photo provided by Angela Perri.)

## WORKING WITH COMPOSITES

Composite research centers on the following: (1) the composite's composition, (2) the composite's context, and (3) the potential for a composite to act as a proxy. Composition largely affects the research questions that can be asked and the methods that are best suited to analysis. Composite context relates to where a composite is collected, what its relative abundance is, and whether it has unique features. Composites may also contain DNA that allows for the proxy study of humans or nonhuman organisms that have similar or equal cultural importance to that of humans.

### Sediment

DNA in sediment (Figure 1a) is often from small fragments of bone and feces, with additional DNA from urine, hair and skin, plant matter, and other discarded organic material (Massilani et al. 2022; Pedersen et al. 2015). DNA from skeletal material may also diffuse into surrounding sediments (Sarhan et al. 2021). This results in sediment containing aDNA that likely represents the floral and faunal environment at the time the sediment was created, with traces of human aDNA depending on context. First analyzed archaeologically by Willerslev and colleagues (2003), sediment aDNA (sedaDNA) has been used primarily in environmental reconstruction. This includes reconstructing floral communities at a single point in time (Gugerli et al. 2013; Jørgensen, Haile, et al. 2012; Jørgensen, Kjaer, et al. 2012; Parducci et al. 2017) and correlating floral, faunal, and environmental shifts (Anderson-Carpenter et al. 2011; Andresen et al. 2004; Birks and Birks 2016; Epp et al. 2015; Seersholm et al. 2020). Sediment has also been used to look for faunal traces to determine what kinds of animal resources a group may have used (Haile et al. 2007; Hebsgaard et al. 2009; Seersholm et al. 2016; Willerslev et al. 2003) and to study extinct species and the timing and circumstances of their extinction (Graham et al. 2016; Willerslev et al. 2003). SedaDNA from burial contexts has been used to study the genetics of fossil hominins and humans, along with various fauna (Gelabert et al. 2021; Sarhan et al. 2021; Slon et al. 2017; Vernet et al. 2021).

Sediment is abundant; it is ubiquitous to archaeological sites, resampling is possible, and very small amounts are needed for genetic analysis. Sediment is commonly collected during excavation and survey, and sediment can easily be subsampled for use in both destructive and nondestructive analyses. Sediment's ability to act as a proxy is particularly useful, because it allows for the study of human DNA without necessitating the destruction of

Ancestral remains. SedaDNA research can be placed into two broad contexts: (1) environmental studies focusing on flora and nonhuman fauna and (2) using sediment as a proxy for Ancestors and other culturally significant organisms. When used for the former, sediment collected from areas with no known human occupation, cultural activity, or cultural significance at any time point is unlikely to contain endogenous human DNA. An example is the sampling and genetic analysis of lake sediments to reconstruct past environments (Anderson-Carpenter et al. 2011; Birks and Birks 2016). Although there is little cause for concern with these kinds of studies given that there is no human association, researchers should be cognizant of whether any of the flora and fauna they detect have cultural significance to the traditional custodians of the land. If so, additional considerations akin to those when working with human DNA may be needed. If sediment is collected from sites with known human occupations, human DNA may be recovered. This is especially true of sites with burials, and human DNA may also be present in layers devoid of visible Ancestral remains. Although sediment can be an excellent alternative to sampling Ancestral remains due to its abundance, human DNA yields from sediment will likely be lower than those gained from sampling Ancestral remains (Sarhan et al. 2021). SedaDNA context can be further complicated by DNA leeching from higher stratigraphic layers (Haile et al. 2007). Recent work has shown that genetically screening sediment from throughout a profile to identify specific taxa and then targeting those taxa from microfossils could mitigate the effects of leeching (Massilani et al. 2022). Alternatively, knowledge of a site's formation and assemblages can be used in conjunction with sedaDNA to reconstruct chronologies.

### Coprolites

DNA in coprolites (Figure 1b) primarily comes from gut microbes, dietary elements, and the defecator (Rose et al. 2015). The aDNA in coprolites provides the identity of the depositing organism and its gut microbiome, along with what it consumed on a given day. Coprolites, which were first genetically analyzed by Poinar and colleagues (1998), are often used to discuss the defecator and its diet. The floral and faunal components of coprolites are useful for reconstructing past diets in both human and nonhuman animals (Boast et al. 2018; Gilbert et al. 2008; Poinar et al. 2001; Wood et al. 2008; Wood, Wilmschurst, Wagstaff et al. 2012; Wood, Wilmschurst, Worthy et al. 2012; Wood et al. 2013), and the microbial DNA has been used to study the gut microbiome (Lugli et al. 2017; Santiago-Rodriguez et al. 2017; Tito et al. 2012;

Wibowo et al. 2021). Several researchers have studied defecator phylogenies and movements across a landscape (Botella et al. 2010; Gilbert et al. 2008; Karpinski et al. 2017; Poinar et al. 2003).

Unlike sediment, coprolites are not common to all archaeological sites and, when found, are present in varying amounts and differing states of preservation. Potential human coprolites are often found in middens or cesspits (Shillito et al. 2020) and may not be collected given that they can be difficult to distinguish from the surrounding sediment. Additionally, human coprolites can be similar in size, shape, and color to the coprolites of nonhuman animals such as canines, making identifications difficult without additional analysis. Although coprolites are primarily a source of dietary and environmental information, they can sometimes act as proxies for their depositing organism. Two broad categories of coprolite analysis are (1) analyses of nonhuman coprolites and (2) analyses of human coprolites. Regardless of their source, coprolites are finite. As with any archaeological or natural resource, sampling should only be done when there are either enough coprolites to leave a portion of the assemblage unanalyzed or when the coprolites are large enough that any sampling would not result in the destruction of the complete coprolite. An example of a large coprolite assemblage is that of Hind's Cave, Texas, where hundreds of coprolites were recovered and only a fraction analyzed (Dean 2006), leaving most coprolites intact. As for coprolite size, fecal material weighing as little as 5 g can be used in a variety of macro, micro, and molecular analyses while still maintaining a voucher sample (Wood and Wilmshurst 2016). Having one to a few coprolites at a site does not mean research cannot be conducted on them, but more care is needed to ensure that multiproxy analyses can take place.

An important consideration with coprolite analysis is that identifying the defecator may require some form of analysis, especially when distinguishing human from canine coprolites at North American sites. If coprolites come from environmental sites or are clearly nonhuman, such as the occasional mislabeled owl pellet, they can be considered as environmental traces. However, as a default, unknown coprolites recovered from archaeological sites should be considered human until additional analyses prove otherwise. In coprolite analysis, this requires either engaging in traditional identification methods by looking at the contents and rehydration liquid (Fry 1985; Reinhard and Bryant 1992) or doing genetic analyses (Borry et al. 2020; Knights et al. 2011; Poinar et al. 2003). As with sediment, preservation of human and other DNA may not be as good as in skeletal material. Even coprolites confirmed as human are not guaranteed to contain analyzable human DNA.

## Birch Pitch and Other Chewed Materials

Birch pitch (for a similar material, see Figure 1c), an adhesive substance used for tasks including hafting, waterproofing, and mending vessels, is a more recent subject of DNA analysis. Small amounts of birch pitch can be common at European archaeological sites (Jensen et al. 2019; Kashuba et al. 2019; Mazza et al. 2006; Ottoni et al. 2021; Rageot et al. 2021; Sykes 2015). Although birch pitch is not found in North American archaeological sites, other plant pitches, adhesives, and gums are found that could contain similar materials and genetic traces as birch pitch (Fox et al. 1995; Langejans et al. 2022). Pitches can be found with tooth and tool marks and, in some cases, fingerprints (Aveling and Heron 1999; Kashuba et al. 2019; Sykes 2015). Plant pitches that

were chewed before use can contain DNA from humans, their diet, and their oral microbiome (Jensen et al. 2019; Kashuba et al. 2019; Lawton 2021; Ottoni et al. 2021). Kashuba and colleagues (2019), the first to genetically analyze birch pitch, and Jensen and colleagues (2019) have shown that birch pitch can provide information about individuals, including their genetic affinities, their oral microbiomes, and their environmental and ecological contexts (Jensen et al. 2019; Kashuba et al. 2019; Rageot et al. 2021; Stacey et al. 2020). Quids—chewed wads of plant matter—are more common to North American archaeological sites than chewed pitches. Quids may display unique morphologies and can contain similar genetic information to pitches. LeBlanc and colleagues (2013) successfully extracted mitochondrial DNA from quids from the southwestern United States, identifying the haplogroups of the chewers.

Even if pitch and chewed materials are plentiful at a site or in a region, the amount that contains clear bite marks, fingerprints, and other distinct morphologies is smaller. Chewed materials can be divided into (1) fragments with no evidence of chewing and (2) fragments with clear evidence of chewing, such as bite marks. Fragments from the former may contain environmental DNA, but they should not be considered as a potential proxy for humans. Chewed materials may contain better preserved human DNA than coprolites and sediment, but due to their rarity, they are not good proxies.

## Dental Calculus

Dental calculus (Figure 1d) is a mineralized biofilm commonly found on teeth that may contain DNA from oral microbes and the individual, along with food remains and environmental particles (Dagli et al. 2015; Preus et al. 2011; Weyrich et al. 2015). De La Fuente and colleagues (2013) were the first to successfully extract and sequence oral microbe DNA from dental calculus. The first high-throughput study of dental calculus DNA was done by Adler and colleagues (2013), who studied questions of diet, pathology, and health. Dental calculus is well preserved, has relatively high DNA yields, and can be less susceptible to contamination compared to composites such as sediment and coprolites (Dagli et al. 2015).

Because dental calculus is found on teeth, it is the only composite discussed here that can be explicitly associated with an individual. Dental calculus can be considered in one of two ways: (1) dental calculus is a biofilm that is separate from the individual, or (2) dental calculus, as it is formed in the body and is on teeth, is part of the individual. Although neither is inherently incorrect, they are two opposing viewpoints that would result in different concerns around studying dental calculus. If dental calculus is viewed as a biofilm, its sampling is not under the same ethical considerations as if the tooth were sampled. Our view is that dental calculus, as it is found on teeth and is directly linked to an individual, should be treated in the same way as the tooth.

## Working with Composites: An Overview

A benefit to working with composites—beyond the information they can provide about diets, environments, microbiomes, and change over time—is that composites sampled from certain contexts can be used to study human genetics while avoiding the destruction of Ancestral remains. The potentially lower genetic

yields from extracting DNA from composites and the possibility that no human DNA is found are acceptable trade-offs to avoid sampling Ancestral remains. However, the sampling and analysis of human DNA from composites cannot be seen as a workaround for collaboration and communication with descendants and other stakeholders (Tsosie et al. 2020).

## ETHICAL ISSUES IN ANCIENT DNA RESEARCH

The ethics of doing research on composites can be informed by the issues that have been raised in discussions surrounding the genetic analysis of Ancestors. At the forefront of this discussion are concerns around studying aDNA, primarily as it relates to historic mistrust, context and interpretation, and access.

### Historic Mistrust

Studying Ancestor remains and belongings, including composites, is integral to archaeological research in the United States, but historically, it has been done with little discussion with or involvement of descendant communities. Through much of its history, archaeology was the purview of Western archaeologists who took upon themselves primary authority to analyze, interpret, and represent cultures with which they were not affiliated (Atalay 2006; Colwell 2016; Colwell-Chanthaphonh et al. 2010; Nassaney 2021; Prendergast and Sawchuk 2018; Tsosie et al. 2020; Van Dyke 2020; Wilcox 2010). These research practices were centered on Western viewpoints and exploited the pasts of descendant groups without taking care to ensure that the knowledge gained benefited them. The lack of recognized descendant autonomy over cultural and physical remains and the failure of archaeologists to include descendants in the research process have contributed to a culture of mistrust between descendant communities and archaeologists. This same mistrust and historic exclusion is seen in genetic research when Indigenous populations faced a lack of control over Ancestor remains, cultural artifacts, and data (Claw et al. 2017; Colwell 2018; Garrison et al. 2019; Handsley-Davis et al. 2021; Malhi and Bader 2015; Tackney and Raff 2019; Tsosie et al. 2020; Van Dyke 2020; Wilcox 2010). Indigenous communities are increasingly involved in the planning and interpretation of archaeogenetic research, but this development is unfolding against the backdrop of hundreds of years of exploitation and marginalization of Indigenous peoples in North America (Nassaney 2021; Van Dyke 2020).

### Context and Interpretation

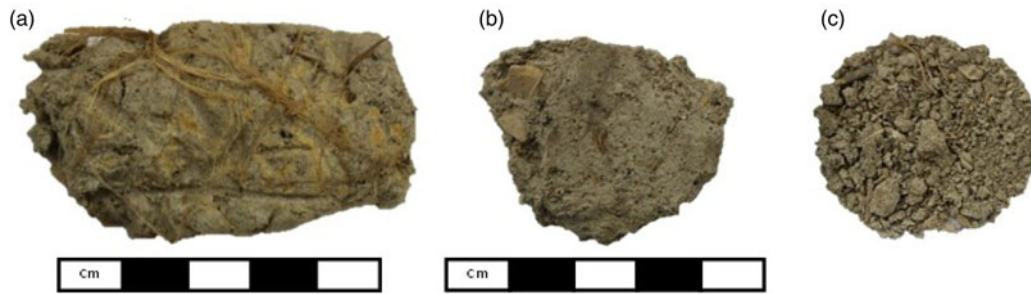
Studies focused on tracing the genetic histories of multiple diverse people groups can be extensive. Although these analyses are useful and necessary for broad characterizations, the detection of trends, and evolutionary studies, researchers do not always engage with the cultural context of or the Indigenous knowledge about the Ancestors they study. This lack of engagement and potential disregard for traditional ways of knowing and cultural data can be common in paleogenetic studies (Crellin and Harris 2020; Fox 2019; Gokcumen and Frachetti 2020; Tackney and Raff 2019). Genetic data that are separated from their cultural context or used in ways not consented to by descendant communities run

the risk of being interpreted in ways that contradict the oral and historic traditions of descendant groups. This disjunct can harm a group's or an individual's sense of self and may remove nuance from data interpretation in favor of simpler, straightforward narratives (Austin et al. 2019; Crellin and Harris 2020; Hakenbeck 2019). Additionally, genetic data have the potential to cause lasting harm to Indigenous peoples by weakening land claims and political standings, stigmatizing groups, and playing into racist ideologies (Garrison et al. 2019; Handsley-Davis et al. 2021; Nassaney 2021).

### Access

Access relates to data stewardship and to the ability to participate in aDNA work. From a research perspective, it is standard practice to publicly share paleogenomic datasets, and in fact, such sharing is often a requirement for publication (Alpaslan-Roodenberg et al. 2021; Anagnostou et al. 2015; Sedig 2019). Few standards exist regarding data format and content. The result is a variety of databases in different formats that have different rules for access and differing amounts of associated metadata (Fox 2019; Fox and Hawks 2019; Powell 2021). However, a greater concern is the frequent lack of access or control that descendant communities have over data generated from their Ancestors (Fleskes et al. 2022; Mackey et al. 2022; Tsosie et al. 2020). Publicly sharing genetic datasets without full collaboration with Indigenous communities largely benefits researchers while potentially harming descendant communities, and not all cultural knowledge or genetic information is meant for public consumption (Carney et al. 2022; Fleskes et al. 2022; Kowal et al. 2023; Nassaney 2021; Van Dyke 2020).

Archaeological materials, including composites, are finite resources. Researchers must rely on either existing collections or new excavations. DNA from newly excavated material is generally better preserved, but additional DNA degradation can occur when samples are stored in less than ideal conditions. This additional degradation can make destructive aDNA analyses (Figure 2) less feasible and less justifiable (Brunson 2019; Brunson and Reich 2019; Fleskes et al. 2022; Fox and Hawks 2019; Pálisdóttir et al. 2019; Pruvost et al. 2007; Sirak and Sedig 2019). Ancestor remains are irreplaceable, and once they are destroyed for genetic analysis, they cannot be reconstructed, studied in nondestructive ways, or returned to descendants. The result of small amounts of available materials, the high costs of genetic research, the need for dedicated facilities, and the specialized knowledge required for genomics is that most studies are performed by a few prominent labs (Austin et al. 2019; Callaway 2017; Fleskes et al. 2022; Fox 2019; Fox and Hawks 2019; Lewis-Kraus 2019; Makarewicz et al. 2017; Mulligan 2006; Pálisdóttir et al. 2019; Prendergast and Sawchuk 2018; Sedig 2019). This has led to a research landscape that too often prevents descendant communities, along with smaller labs and research groups, from accessing materials or engaging in archaeogenetic research without collaborating with or giving research control to one of the larger groups (Lewis-Kraus 2019; Somel et al. 2021). Furthermore, cultural material and genetic samples are often stored in academic institutions or museums that may be far removed from descendants (Colwell-Chanthaphonh et al. 2010; Lippert 2006; Nilsson Stutz 2018; Wilcox 2010). When descendants are not involved in research, they can lack access to their physical past and to the interpretation of that past (Atalay 2006; Handsley-Davis et al. 2021).



**FIGURE 2.** Representation of the destructive nature of composite research: (a) whole paleofecal sample before subsampling; (b) exterior was removed and the sample cut in half. Subsamples for aDNA analysis were collected from the center and homogenized; (c) half the remaining material was disaggregated for macroremains and microremains analysis. (Photos provided by Taryn Johnson.)

### A Cautionary Tale

Researchers extracted DNA from nine ancestors interred with funerary objects from Pueblo Bonito in Chaco Canyon with the goal of adding genetic data to archaeological debates about the role of kinship in the development of complex societies. They found that the individuals shared mitochondrial genomes and represented an elite matriline. The researchers further determined the relatedness of six of the Ancestors by genotyping single-nucleotide polymorphisms (SNPs) from their nuclear DNA (Kennet et al. 2017). Consultation of local tribes was not legally required because the Ancestors were considered culturally unaffiliated by their housing institution. This study now serves as an example of how a lack of descendant collaboration in research can cause harm. Claw and colleagues (2017) brought up three main concerns with the study: (1) tribal groups were not consulted, (2) some data descriptions were culturally insensitive, and (3) the researchers did not consider how the study might affect descendants. Tribal knowledge also includes descriptions of matrilineal structures, which would have aided interpretation and put the findings in a broader cultural context. The study had the added effect of degrading long-term collaborative relationships between descendant communities and regional archaeologists (Claw et al. 2017; Cortez et al. 2021; Van Dyke 2020).

## FRAMEWORKS FOR PLANNING COLLABORATIVE RESEARCH

As with all archaeological research, composite aDNA studies should start with a clear research question, and collaborators need to consider whether DNA is needed to answer it (Figure 3). Would genetic data give new information or provide additional evidence in support of an existing theory? Will the findings be novel? Can the results be gained only through DNA analysis? Could less destructive methods be used? Destructive sampling should only be done if the possible results are worth destroying the composite and if the generated data could benefit community partners without causing harm (Fox and Hawks 2019; Handsley-Davis et al. 2021; Sirak and Sedig 2019). Composite work is multiproxy and should not be limited to genetic analyses. Composites can contain macrobotanical and faunal remains, pollen, starch, phytoliths, parasites, proteins, fatty acids, and other biomolecules in addition to DNA. All these contents can provide valuable information

about how an individual interacted with the world through health, food, and the environment. Multiproxy composite analyses allow for individuals to be placed in a larger cultural context, and they likely require interdisciplinary teams. Researchers need to be open with community partners about the full range of analyses that could be done using composites and discuss if nondestructive or less destructive methods of analysis are better suited to the research.

In terms of research, aDNA work, even on composites, is destructive. Composites should be fully documented and conservatively subsampled (Alpaslan-Roodenberg et al. 2021; Brunson and Reich 2019; Pálsdóttir et al. 2019; Sirak and Sedig 2019). Even if nongenetic analyses are not planned at the time of sampling, responsible composite research should involve subsampling for other analyses along with DNA subsampling. Sampling once allows for multiple analyses to be conducted without repeated handling and destruction of the composite (for an example of a subsampling procedure, see Blong et al. 2023). Documentation for morphologically indistinct composites such as sediment and dental calculus may include provenience, subsample weight, and composition. Additional documentation is needed for morphologically distinct composites such as coprolites and birch pitch. Coprolites, for example, can be photographed, weighed, measured, and qualitatively described using traits such as color, shape, state of preservation, taphonomic modifications, and presence of inclusions (Jouy-Avantin et al. 2003; Wood and Wilmshurst 2016). Birch pitch may be physically described, photographed, scanned, or used to make a mold (Jensen et al. 2019; Kashuba et al. 2019). Any DNA extraction should be done using established protocols developed for the composite type (Epp et al. 2019; Hagan et al. 2020; Jensen et al. 2019).

At a bare minimum, research plans should align with the rules and regulations of where the composites are from and where the research is being conducted; this includes regulations from the local to national level (Alpaslan-Roodenberg et al. 2021; Claw et al. 2018; Kowal et al. 2023; Pálsdóttir et al. 2019). However, composites and the Ancestor DNA they may contain are often excluded from current guidelines and discussions, which predominantly focus on genetic analyses of Ancestral remains (Makarewicz et al. 2017; Squires et al. 2019). This exclusion does not mean that composites are a way around collaboration, ethical research practices, and legal requirements. Additionally, although legal and biomedical frameworks in the United States are not



**FIGURE 3.** Example research flow for planning composite genetic research. First, a research question is established, and whether DNA analysis is needed to answer it is considered. Researchers collaboratively engage with stakeholders, integrate stakeholder goals into the research plan, and discuss data dissemination. The sequencing method can be chosen based on the type of data needed and whether stakeholder consent was given.

comprehensive and do not offer aDNA or composites the same protections and regulations as Ancestor remains and living subjects (Fleskes et al. 2022), they can act as a baseline when planning research. In a fully collaborative, open framework, community partners are included in the creation and implementation of the research plan, and their research goals are equally considered to those of the researchers (Alpaslan-Roodenberg et al. 2021; Fox and Hawks 2019; Matisoo-Smith 2019; Sirak and Sedig 2019; Wagner et al. 2020). This improves research transparency, expands research goals, and helps establish relationships of trust between researchers and other stakeholders (Claw et al. 2018; Handsley-Davis et al. 2021; Tackney and Raff 2019).

### Legal and Biomedical Frameworks: NAGPRA and the Belmont Report

In the United States, the Native American Graves Protection and Repatriation Act of 1990, or NAGPRA, provides legal protections

for human remains, funerary objects, sacred objects, and objects of cultural patrimony. This legislation rarely applies to composites and does not prohibit DNA analysis (Fleskes et al. 2022; Van Dyke 2020), but the consultation process that NAGPRA inspires (but, significantly, does not require) can serve as a guide during the planning phase of a research project. The concept of stakeholder engagement, which involves early and frequent communication with stakeholders, is central to the NAGPRA document (United Nations Evaluation Group 2017). The basic consultation steps involve identifying stakeholders, sharing research information, discussing how the consultation process should proceed, and disseminating data at the conclusion of the project (Bureau of Land Management 2016; Monette et al. 2018).

A related biomedical framework is the standardized treatment of humans by biomedical researchers. The *Belmont Report*, a response to the infamous “Tuskegee Study of Untreated Syphilis in the Negro Male in the United States,” emphasizes that

researchers need to treat people with respect and to avoid causing harm. The report provides a framework for the treatment of living subjects centered on three ethical principles: (1) respect for persons, (2) beneficence, and (3) justice, which in practice calls researchers to respect personal autonomy, minimize harms, and do research in ways that benefit affected communities (National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research 1979). Another principle outlined in the *Belmont Report* is voluntary participation. The deceased cannot give informed consent, so for ancient genetic analyses, it falls to descendant communities to consent to research. If the letter of the law is followed, composite research is rarely subject to consultation. Therefore, although NAGPRA and the *Belmont Report* can provide some guidance, their specific focus on living humans and Ancestor remains necessitates the development of an ethical framework specifically for composites.

## Collaborative Frameworks

Stakeholders are the individuals, communities, museums, and other institutions that have cultural or professional connections to a study (Alpaslan-Roodenberg et al. 2021; Pálsdóttir et al. 2019). We recognize that descendant communities often have strong, vested interests in aDNA research extending beyond those of generic “stakeholders.” However, for ease of conversation, we are including them within the broader category of stakeholders in this discussion. Stakeholders may include descendant populations and local, state, national, and tribal entities, in addition to archaeologists, other researchers, and curating institutions. When researchers work with Indigenous North American materials, the stakeholders include the modern tribal or Indigenous groups, including both lineal and cultural descendant communities. Collections managers and other museum professionals who are often involved in curating composites, who engage in conversations with the descendant communities whose objects they house, and who approve research proposals involving destructive analysis should also be included in conversations about the potential benefits and limitations of proposed research.

Composite research—and more broadly, archaeology—is inherently collaborative work that integrates multiple perspectives and methods. Working with stakeholders on an egalitarian basis creates space for combining multiple viewpoints that go beyond the Western framework and leads to better science (Colwell-Chanthaphonh et al. 2010; Nilsson Stutz 2018). Studies need to be considered within their own contexts, and one viewpoint or a single approach cannot give a complete picture of the past. Opening the work to differing viewpoints, ways of knowing, and cultural knowledge leads to a more nuanced, fuller view of the past that shifts the focus of research back onto human questions (Colwell 2016; Colwell-Chanthaphonh et al. 2010). Inclusivity further allows archaeology to both contend with and build on its past and inform contemporary debates and solutions (McAnany and Rowe 2015; Nilsson Stutz 2018).

Involved communities should include those who are culturally affiliated and those with links to the Ancestors, and communication should occur throughout a research project. This means that communities and local research groups are equal partners in all parts of the research process. In cases where descendant communities cannot be identified, where there are conflicts, or where community consent is withdrawn, research may need to stop

(Fleskes et al. 2022; Kowal et al. 2023). Researchers who decide to move forward with research need to explicitly state why moving forward is justifiable. The goals of engagement should be focused on collaboration and building competency (Claw et al. 2018; Tackney and Raff 2019; Wagner et al. 2020). Researchers should assess how composites are viewed by the relevant communities and ensure that all project terminology, documentation, and goals align with their belief structures. All aspects of research should be discussed before and throughout a project (Alpaslan-Roodenberg et al. 2021; Garrison et al. 2019; Matisoo-Smith 2019; Wagner et al. 2020), and discussion should occur before any sampling is done (Tackney and Raff 2019). Communicating openly and on an equal basis with communities and individuals who have cultural and historical connections to research opens the door for true collaboration.

Including community members enables them to educate professional researchers about their past and their concerns about research plans (Atalay 2006; Colwell 2016; Colwell-Chanthaphonh et al. 2010; McAnany and Rowe 2015). This leads to a shift from archaeological gatekeeping to a more collaborative framework that has modern relevance, that benefits descendant communities, and that has a wider audience. Giving equal consideration to other views can lead to new research avenues and reveal theoretical and personal biases in data interpretation while putting materials in context and bringing broader understanding (Brunson and Reich 2019; Colwell 2016; Colwell-Chanthaphonh et al. 2010; Fox and Hawks 2019; Kiddey 2020; McAnany and Rowe 2015; Nassaney 2021).

## DATA GENERATION

### A Quick Guide to Genetic Sequencing Methods

Different sequencing methods will yield different types and amounts of information, allowing collaborators to choose methods that are best suited to the goals of the project. For any aDNA project using composites, the goal should be to choose methods that result in the least amount of destruction with the greatest yield of the target DNA. Once DNA has been extracted from a composite, it may be amplified and sequenced. Amplification involves copying extracted DNA to create millions of new copies; sequencing refers to identifying the order of bases (adenine, guanine, thymine, cytosine) in each strand of DNA. Ancient DNA methods are summarized in detail elsewhere (Liu et al. 2022; Orlando et al. 2021). Here, we briefly describe Sanger sequencing, metabarcoding, and shotgun sequencing to provide a short introduction for individuals unfamiliar with genetic analyses.

*Sanger Sequencing.* Sanger sequencing is not commonly used in composite genetic research, although there are cases where it may be useful (Linderholm 2015). Examples include, but are not limited to, quickly and affordably testing for the presence of certain organisms before engaging in more costly sequencing methods, identifying macroremains within composites, and, in the case of coprolites, identifying possible sources. In Sanger sequencing, researchers identify a target gene in an organism and create primers, or short strands of DNA, that match the targeted gene. When these primers are used in amplification, only the target is amplified and sequenced (Sanger and Coulson 1975). The cost per sequencing run is negligible, but it also yields the least amount of data. Sanger sequencing is the least time

intensive and requires no specialized bioinformatics training, because it only yields a single genetic sequence. Although not discussed here, methods including DNA capture techniques and zooarchaeology by mass spectrometry (ZooMS) can be a more cost-effective, less destructive, more accurate way to identify specific composite contents than Sanger sequencing (Liu et al. 2022; Richter et al. 2022).

**Metabarcoding.** Metabarcoding is widely used to monitor genetic biodiversity (Bohmann et al. 2022), and the method is useful for identifying specific groups of organisms in composites. Like Sanger sequencing, metabarcoding involves targeted amplification and sequencing but uses universal primers. Universal primers match a target gene that is common to a group of organisms. Two examples of universal primers are *trnL*, which targets plants, and *12sv5*, which targets vertebrates (Pedersen et al. 2015; Staats et al. 2016). A study using *trnL* will not amplify human or other animal DNA, whereas a study using *12sv5* may amplify human DNA along with that of other vertebrates. Metabarcoding studies are often based on ubiquity, meaning that amplified human DNA is not likely to yield any information other than human presence. Additionally, when human DNA is not specifically targeted, the human DNA is often considered contamination and removed from the dataset before conducting analyses (Alsos et al. 2018; Giguet-Covex et al. 2014; Seersholm et al. 2020). DNA metabarcoding is more costly than Sanger sequencing per run—although it is cheaper per base pair—and requires more intensive bioinformatics processing given that multiple samples are often pooled to be sequenced simultaneously (Bohmann et al. 2022). For example, metabarcoding has utility in analyses such as environmental reconstruction from sediment. The results will be a list of present taxa, and samples from different stratigraphic layers can be sequenced in tandem. The basic steps, as described by Mathon and colleagues (2021), are demultiplexing, dereplication, quality filtering, error removal, and taxonomic assignment. These mean that DNA from individual samples is first separated, after which highly similar DNA is grouped together and represented by a single sequence. Quality filtering involves removing sequences from the dataset that are the wrong length or have ambiguous bases, and error removal removes any sequences that were formed via errors in amplification or sequencing. Taxonomic assignments are made by comparing the resultant DNA sequences to reference genetic databases (Mathon et al. 2021). Metabarcoding can yield millions more reads than Sanger sequencing, and the DNA is best used for taxonomic identifications.

**Shotgun Sequencing.** Shotgun sequencing allows researchers to both identify composite contents and analyze the full genomes of those contents. Unlike the previous methods, shotgun sequencing is not targeted. Ancient DNA is made up of small DNA fragments from across a genome, and in composites, this means that the DNA comes from the genomes of several organisms. Whereas the targeted methods will pick out only certain fragments, untargeted methods result in the sequencing of a representative sample of all fragments (Knapp and Hofreiter 2010). Because of this, shotgun sequencing will sequence any human, faunal, floral, and microbial DNA in a composite even if the researcher does not plan on analyzing it. Of the three sequencing methods discussed, shotgun sequencing is the most used method in composite research because it yields the most data; however, it costs more per run than other methods (although less per base pair) and requires the

most data processing. Given that shotgun sequenced datasets are composed of fragments of DNA from across the genomes of multiple organisms, the DNA sequences first need to be assembled into their respective genomes; this entails aligning the fragments using existing genetic datasets as reference. After assembly, the DNA can be partitioned into groups for further analysis. For example, a paleofecal dataset may be divided into DNA belonging to humans, dietary taxa, parasites, and microbes. Each group of data can then be separately processed and analyzed according to the goals of the project.

## DATA DISSEMINATION AND CURATION

As shown in the above section, genetic data from composites could be in the form of single sequences belonging to one organism or individual, a collection of representative gene sequences belonging to multiple organisms or individuals, or a range of sequences from across genomes belonging to multiple organisms or individuals. Researchers should develop and implement a plan at project outset for managing data, reporting results, and storing data. Discussions about the dissemination of this genetic data center around the concepts of data sharing and data sovereignty (Alpaslan-Roodenberg et al. 2021; Carney et al. 2022; Claw et al. 2018; Fox 2019; Pálsdóttir et al. 2019; Sirak and Sedig 2019; Wagner et al. 2020). Open data sharing, where all information and results are publicly available, may be a requirement of publication. Journals including *PLoS ONE*, *Science*, and *Nature* require that data be made available upon publication, whereas journals such as *PNAS* strongly encourage data publishing but allow concessions for ethical concerns. Open data sharing allows for reanalysis and lessens the need to resample, but the benefits of publicly available data need to be weighed against the harm communities may face if sensitive information is published. A response to this need is increased Indigenous data sovereignty, whereby Indigenous peoples maintain ownership over data and moderate both access to and use of the data by researchers and communities (Carney et al. 2022; Garrison et al. 2019; Mackey et al. 2022). Information may be shared in Indigenous databases, with neutral third parties, in open-access databases, or in restricted-access databases (Alpaslan-Roodenberg et al. 2021) depending on the needs of stakeholders.

Results should be written in a manner that respects the people being discussed and should be shared in a location and format that is accessible (Alpaslan-Roodenberg et al. 2021; Tackney and Raff 2019; Tsosie et al. 2020). Stakeholders should be involved in the review process and be able to request both redactions and technical corrections on manuscripts (Fleskes et al. 2022). Although results are likely to be published in peer-reviewed journals, dissemination of results can go beyond academic publications. Plans for data sharing can include publishing results on an organization's website, writing social media updates, giving public talks, agreeing to interviews about the work, and presenting at conferences.

## CONCLUSION

Composites can be used to answer a variety of research questions regarding human identity and their interactions with dietary and



environmental landscapes. Although composites can additionally act as proxies, their analysis cannot be seen as a workaround to collaborating fully with descendant communities and other stakeholders. The current discourse about ethically working with human remains serves as an excellent starting point for discussing composite work, given that established frameworks, ethical principles, and guidelines can be applied directly to research on composites from the United States. These frameworks call for establishing clear research plans and maintaining open, honest communication. Only research that has been fully discussed with stakeholders should be performed, and care should be taken to include stakeholders as full and equal collaborators throughout the project. Transparent, well-thought-out studies can help continue, improve, and forge new relationships between stakeholders, researchers, and the broader public, leading to fuller interpretation of data and additional research opportunities.

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## Competing Interest

The authors declare none.

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