DNA METABARCODING AND MACROREMAINS ANALYSIS OF MIDDLE AND LATE HOLOCENE PALEOFECES FROM BONNEVILLE ESTATES ROCKSHELTER, NEVADA

A Thesis

by

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MASTER OF ARTS

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ABSTRACT

The genetic and macrofossil composition of paleofeces from Bonneville Estates Rockshelter (BER) can aid environmental and dietary reconstruction, as the dietary contents of coprolites change as environmental conditions shifted from hot and dry in the Middle Holocene to cool and moist in the Late Holocene, and as the distribution of food resources shifted locally. To analyze the potential shift in taxonomic diversity and genetic biodiversity present in prehistoric diets of the human occupants over this transitionary period, ancient DNA was extracted and macroremains sorted from ten paleofecal samples from Bonneville Estates Rockshelter, eastern Nevada, USA. Identifications of floral and faunal contents were established at the lowest possible taxonomic levels, and results were compared to analyze how Archaic diets may have shifted through time, from about 7000 to 1000 years ago. Additional comparisons were made between the molecular and macroscopic results to determine the differences in the kinds of traces found in each. Results suggest a strong reliance on dryland resources, especially small seeds, throughout the rockshelter's occupations, with some integration of more wetland resources. Additionally, DNA metabarcoding and macroremains analysis display complementary utility, as there was little overlap between the traces found in each. Further research will be done to determine if additional shifts, be they cultural, populational, or otherwise, occurred alongside the dietary changes. Broader applications of this study consider the effect of climate change on floral and faunal

populations and how humans interacted with the biotic parts of their environment, leading to greater understanding of past and present human ecology.

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All other work conducted for the thesis was completed by the student independently.

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CHAPTER I

INTRODUCTION

The molecular and macroremains of paleofeces, also known as coprolites, can provide direct information regarding a depositing organism's diet, health, and environment (Fry 1970, 1976; Reinhard and Bryant 1992, 2008; Shillito et al. 2020). This utility is due in large part to fecal composition. Macroremains in feces can include whole or fragmented seeds and other plant parts, charcoal, marine and terrestrial faunal elements, avian and insect remains, hair, and fibers (Fry 1970; Reinhard and Bryant 1992; Shillito et al. 2020). Molecular remains show traces of the depositing organism, microorganisms, and dietary contents of a sample (Rose et al. 2015; Shillito et al. 2020). Though outside the scope of this thesis, contents can also include plant microremains such as pollen, starch, and phytoliths, while additional biomolecular elements such as proteins and lipids may also be present (Shillito et al. 2020).

Quantification of Contents

Due to their varied contents, coprolite research is inherently multiproxy; analyses of multiple components may be necessary to see the whole dietary range present in a sample (Blong et al. 2020; Blong and Shillito 2021; Shillito et al. 2020; Wood and Wilmhurst 2016). However, while the contents suggest intentional ingestion of certain food resources, quantifying those materials and their relative importance in the diet can be difficult. Coprolites are not always homogeneous, either within a sample or between

samples from similar contexts. Items move through the digestive tract at different rates, meaning one coprolite does not directly correlate to one meal (Fry 1970,1976, Rose et al. 2015). Additionally, materials undergo changes during processing and digestion. How a dietary item is processed and the nature of the part that is consumed can affect preservation of both the molecular and macroremains (Fritz and Nesbitt 2014).

A small number of studies have looked at how taphonomic processes in the human gut affect preservation (Butler and Schroeder 1998; Calder 1977; Jones 1986, 1990; Nicholson 1993). The bulk of these focus on the digestion of fish remains, finding that many were either fully digested or so altered as to render them unidentifiable (Butler and Schroeder 1998; Jones 1986, 1990; Nicholson 1993). Identifiable bones or fragments were generally more robust than their digested counterparts (Butler and Schroeder 1998; Jones 1986, 1990; Nicholson 1993). An analysis of New Zealand Maori coprolites showed that materials with high amounts of undigestible keratinous, siliceous, or cellulose materials are most likely to leave identifiable traces (Calder 1977). However, as stated by Nicholson (1993), the results of these kinds of studies can change depending on the health of the individual and how a food item is prepared and masticated.

Generally speaking, the results of taphonomic analyses suggest that softer, more digestible materials such as meats, fruits, and legumes may be less visible in a paleofecal assemblage than harder, less digestible items such as bone, seeds, and plant fiber. Furthermore, processed materials such as those that are ground, made into meal, cooked, or heavily chewed may be harder to detect in coprolites than less processed or raw

materials (O'Meara 2014). Therefore, the frequencies of identified components could be more related to differential preservation and processing than to amount of use or relative importance. The greatest barrier to quantification, however, is that not enough is known about how the human gut digests different materials (Shillito et al. 2020).

Because of this, coprolite analyses are primarily focused on ubiquity, but attempts at quantification have been made. As described by Bryant and Dean (2006), researchers have used percentage-estimates, given abundance ratings of items, summarized quantity according to weight, and done visual estimates of abundance of certain materials. However, each chosen method favors different items, be they heavier, bulkier, or more numerous (Bryant and Dean 2006). Abundance measures are better suited to analyses within samples than between them, and weight-based and visual methods are used in this thesis to aid in the comparison between DNA and macroremains in individual samples, while ubiquity is used to compare contents between samples and components.

Identifying the Defecator

A central concern of coprolite research is identifying the defecator as human. Other animals may have been present or used the same sites as humans, meaning there is a chance that fecal deposits are from non-human animals. This is especially a concern at sites with human-dog (*Canis* sp.) cohabitation; dogs may have consumed the same kinds of food as humans, ate human feces, or both. Because of this mutual omnivory, dogs and humans can have similar feces in terms of morphology and contents (Guiry et al. 2012;

Shillito et al. 2020) making their differentiation essential. Methods for identification generally fall into two categories: traditional methods relying on physical characteristics and visible contents, and molecular methods relying on biomolecular contents.

Traditional Identification Methods

Traditionally, identification relied on qualitative data such as size and shape, color, and presence of inclusions (Fry 1970; Gilbert 2008; Reinhard and Bryant 1992). However, this can be difficult as human coprolites are not morphologically consistent. They come in various shapes and sizes, have drastically different contents, and can be differentially preserved, compressed, or fragmented (Reinhard and Bryant 2008; Shillito et al. 2020). The morphology of feces can change for an individual and at a site depending on what was eaten on a given day and can also change based on the health and age of the person (Rose et al. 2015; Shillito et al. 2020). Possible human coprolites have also been identified by their provenience; they may be found near known habitation areas or in waste-disposal contexts such as middens and latrines (Reinhard and Bryant 1992; Shillito et al. 2020). However, coprolites have historically been overlooked at archaeological sites; the difficulty of identifying them *in situ* can lead to them being discarded, destroyed while screening, or not excavated (Fry 1970; Reinhard and Bryant 2008; Shillito et al. 2020).

Another qualitative trait is the color, translucency, and smell of the rehydrating liquid. Generally, human feces turn trisodium phosphate solution dark brown or black and opaque. This may be accompanied by a strong fecal smell. Carnivore and herbivore

feces generally turn the liquid a translucent pale yellow or brown, with herbivore feces giving off a musty smell (Fry 1976; Shillito et al. 2020). While studies largely support this, not all fecal samples react in this way. Some human coprolites do not turn the liquid dark or give off strong odors, while some herbivore and carnivore feces do. Thus, although color and smell may be a useful guide to which feces are human, they are not definitive (Fry 1976).

Another traditional method considers coprolite contents, primarily the macroremains. Human coprolites are expected to represent a wider dietary breadth than those of other organisms; however, this is not always the case as the diets of foraging groups could greatly vary between days (Reinhard and Bryant 1992). Samples that contain either a variety of known dietary elements or large amounts of fewer dietary elements could be considered human. However, the presence of high amounts of large, undigestible faunal elements such as bone and hair could be indicative of a carnivore (Albush 2010; Witt et al. 2021, Wood et al. 2016). Contents should be used in conjunction with other traditional methods. An extension of this is looking at the parasitical contents, where human coprolites are identified by the presence of human-associated parasites (Fry 1976). The presence of dog-specific parasites has been used to determine a non-human origin (Hagan et al. 2020; Jimenéz et al. 2012; Reinhard and Bryant 2008), just like the presence of only human-specific parasites could be indicative of a human origin (Fry and Moore 1969; Moore et al. 1969; Reinhard 2016; Søe et al. 2015).

Molecular Identification Methods

Molecular identification methods initially focused on mitochondrial DNA (mtDNA). For example, Poinar et al. (2001) analyzed mtDNA from three coprolites found in Hinds Cave, Texas. The presence of haplogroups affiliated with contemporary Native Americans was used to indicate a human origin for the samples. The same method has also been used to identify non-human sources. Hofreiter et al. (2000) compared the mtDNA from five Gypsum Cave, Nevada, samples to a DNA sequence from an extinct ground sloth (*Nothrotheriops shastensis*) bone to confirm their sloth origin. Generally speaking, this form of identification is largely based on ubiquity. Human coprolites are those containing identifiable, endogenous human DNA. The sources of non-human coprolites, which may be more morphologically consistent and distinct from human, are confirmed by the presence of the suspected species' DNA.

However, as stated in section 1.1, the presence of dogs and humans at a site could prevent or obfuscate source identification. One case is given by Gilbert et al. (2008). Three of the six coprolites analyzed from the Paisley Five Mile Point Caves contained both human and dog DNA and were identified as human; the presence of dog DNA could be explained by the presence of canid bones at the site, or dogs may have been consumed by humans, occupied the space when humans were absent, or urinated directly on human coprolites. Depending on context, samples containing both human and dog mtDNA could be attributed to either species.

In the last decade, the gut microbiome has shown utility in defecator identification, partially due to the wealth of information known about human and dog

microbiome composition (Schnorr et al. 2016). Witt et al. (2021) used SourceTracker (Knights et al. 2011) to confirm canine sources for coprolites by comparing the samples' microbial contents to published human and dog microbiomes. The same method was used by Hagan et al. (2020) to confirm the sources of one dog and two human coprolites from Cueva de los Muertos Chiquitos in Mexico. Further work by Borry et al. (2020) in the development of CoproID confirms that microbiome composition and host DNA from shotgun sequenced data sets offer a reliable way to infer the fecal source while avoiding the dog versus human concerns. However, not every coprolite study involves or requires shotgun sequencing, showing a continuing need for traditional and amplicon-based identification methods and tests of their efficacy.

Multiproxy Coprolite Analyses

Multiproxy studies on the dietary contents of paleofeces have been performed on both human and non-human samples. Some of the most well-studied non-human coprolites are those of the New Zealand moa (*Avies* sp., *Dinornithiformes* sp.), a now-extinct ratite. Moa coprolites and the specific species that deposited them have been identified through targeted analysis of moa DNA, while floral DNA, macrofossils, and pollen have been used to reconstruct their diets and ecology (Wood et al. 2008, 2012a, 2012b, 2013). Dietary data show that, unlike previously hypothesized, moa grazing was not restricted to forest habitats. They consumed a variety of herbs, shrubs, and trees, and later research supported moa consumption of fungal resources as well (Boast et al. 2018). It was seen that moa diets varied more between habitats than between species,

and dietary data could be used to study additional questions regarding seasonality and the broader interactions between moa and their environment (Wood et al. 2008, 2013).

A second example is that of dog coprolites from the Janey B. Goode and East Saint Louis sites in the American Bottom, Illinois (USA). Researchers analyzed the macroremains, stable isotopes, and DNA to reconstruct diets. The macroremains and DNA were used to detect specific dietary items, the DNA was further analyzed to detect trends in the gut microbiome, and the stable isotopes were used to determine overall dietary trends. Through combination of these methods, it was found that the dogs had a wide dietary breadth focused on local plant foods and various wetland resources. Because of the dogs' proximity to humans and the dearth of human remains at the site, the results could serve as a proxy for human diets (Witt et al. 2021). A further multiproxy study is that of Wood et al. (2016), who analyzed the microscopic and DNA contents of coprolites from Polynesian dogs. DNA was used to confirm a canine depositor, and the microscopic contents displayed a diet of primarily marine elements such as fish and shellfish, with additional consumption of plants. Because the detected plants were both native and cultivated, their diets likely overlapped with the Maori and may serve as a proxy. The contents can also shed light on dietary behaviors such as what kinds of resources may have been regularly utilized (Wood et al. 2016).

When looking at human coprolites, early multiproxy studies combined macroremains and pollen analysis. A comparative study of these remains from coprolites found in the Glen Canyon region of Utah by Callen and Martin (1969) showed that the two lines of evidence do not necessarily overlap. For example, the macrobotanicals in

one of their samples, coprolite 7, were predominantly cheno-ams (taxa belonging to Chenopodiaceae), *Cucurbita*, and *Agave*, while the pollen contents were almost entirely from grasses. They suggest the macrobotanicals provide more dietary resolution, while pollen data can supplement the macrofossils and reveal additional dietary flora. A study of 19 Hidden Cave (Figure 1) coprolites by Rhode (2003) also focused on macrobotanical and pollen data, with the addition of steroid analysis. The samples date to early and late periods of occupation during the Late Holocene, and contents revealed an initial diet heavily reliant on wetland resources including bulrush (*Schoenoplectus* sp.), cattail (*Typha* sp.) pollen, fish, and waterfowl. Samples from the later period showed increased reliance on desert resources through expanded small seed diversity and decreases in cattail pollen. The steroidal content showed the depositors, and users of the cave, were likely female which could suggest gendered differences in the use of Hidden Cave.

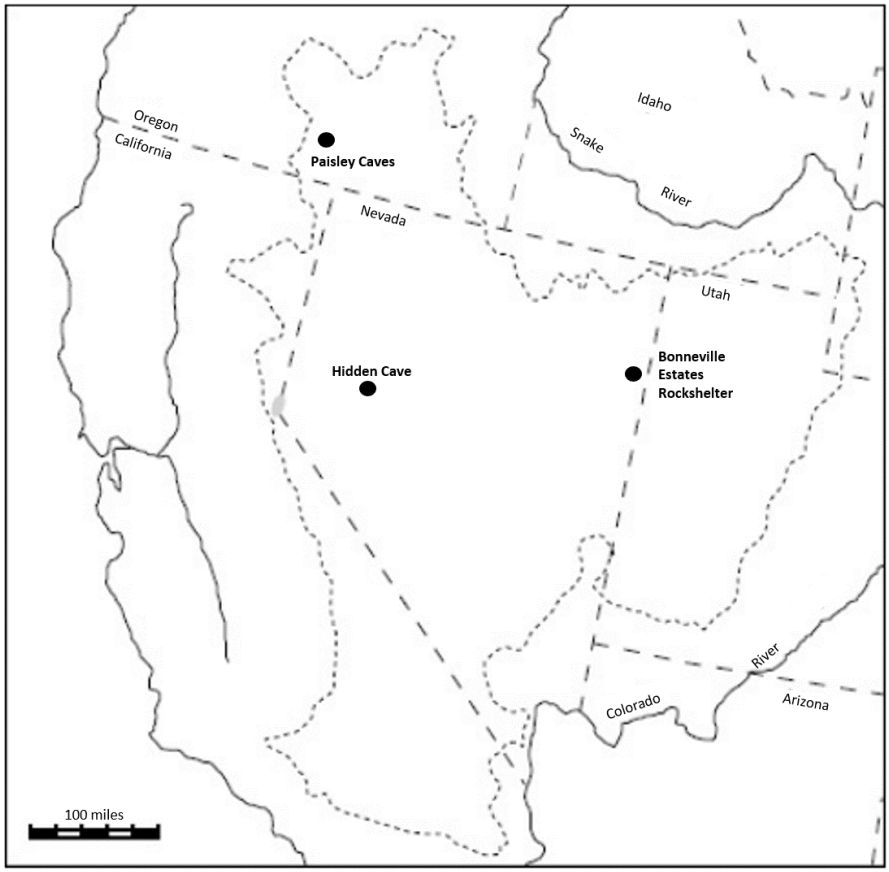

Figure 1 Map of Great Basin sites mentioned in text after Grayson (2011)

The analysis of Younger Dryas/Early Holocene coprolites from the Paisley Caves, Oregon, (Figure 1) by Blong et al. (2020) focused on pollen, phytolith, macrobotanical, faunal, and insect remains. Using traditional methods, samples were identified as human and their contents displayed evidence of a broad subsistence strategy focused on both wetland and dryland resources including seeds, fruits, monocots, dicots, flowering plant parts, small and medium mammals, birds, hares (*Lepus* sp.), rodents, fish, and insects. While faunal remains and hard floral remains such as seeds were found in the macroremains, other dietary elements were identified using pollen and phytoliths.

The samples also provide some of the earliest evidence for the consumption of whole rodents, ten-lined June beetles (*Polyphylla* cf. *decemlineata*), and desert stink beetles (*Elodes* sp.) in the Great Basin. In addition, the lack of evidence for food storage in the region and the seasonality of the plant resources suggest the caves were likely occupied in the summer and fall.

As is shown by the above examples, multiproxy coprolite analyses can provide a wide range of information regarding the depositor and their interactions with the broader environment. Specifically, depending on the methods chosen, contents can shed light on depositor biology, show differences in habitat exploitation, detect unknown dietary elements, track dietary change over time, suggest seasonal use of sites and resources, and aid in reconstructing the diets of past human and animal populations.

Thesis Outline

This thesis is composed of five chapters. Chapter 1 provides an introduction. Chapter 2 contains a description of the samples and the methods used for analysis. This includes a description of Bonneville Estates Rockshelter and the environmental, temporal, and cultural contexts of the samples. The methods include a description of sample selection, subsampling, and analysis of the DNA and macro-contents. Chapter 3 reports results of ancient DNA and macroremains analysis, while Chapter 4 directly compares the two utilized methods and places the findings in the context of other similar studies in the region as well as previous analyses of other materials accomplished at

Bonneville Estates. Chapter 5, finally, presents the overall conclusions of the study and a discussion of future work.

CHAPTER II

MATERIALS AND METHODS

Ten coprolite samples from Bonneville Estates Rockshelter (BER) were selected for analysis (Figure 1). Half are from Stratum 14, three are from Stratum 3b, and two are from Stratum 3a (Figure 2, Table 1); these are associated with components V, III, and II, respectively (Table 2). Coprolites from these strata were sampled as they are associated with human occupation, there is available supplementary dietary data for these time frames, they represent different and unique environmental and/or cultural contexts, paleofeces from these strata contain visible macroremains, and they are well-represented components of the overall paleofecal assemblage. Individual samples were selected according to the following criteria: they had not been previously analyzed, they were sufficiently large for additional analyses and curation after subsampling, and in appearance they matched expectations of human coprolites. In addition, a previous study on BER coprolites showed that samples from the site contained a variety of well-preserved, identifiable dietary elements that correlated to known environmental and dietary data (Albush 2010).

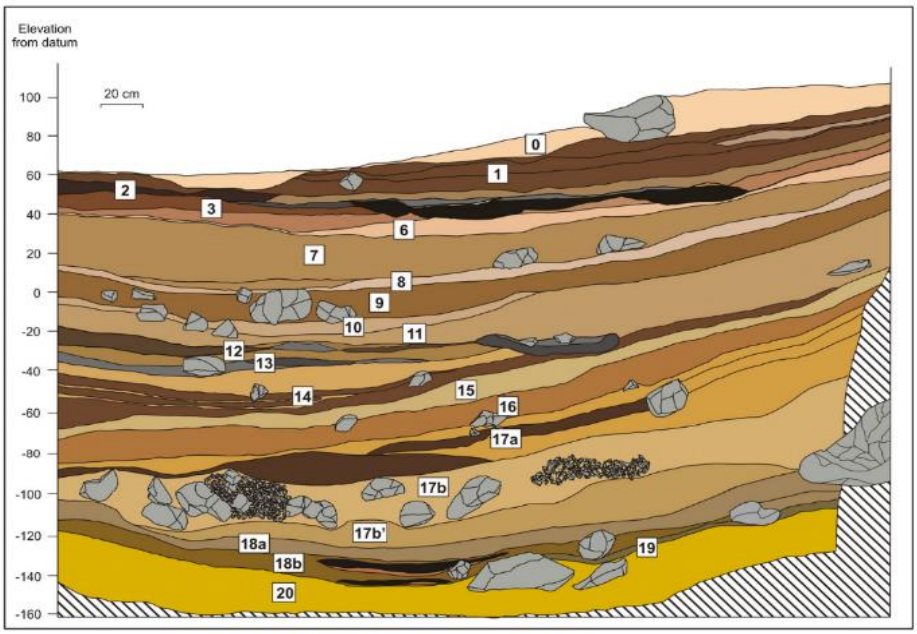

Figure 2 West Block stratigraphic profile from Bonneville Estates Rockshelter (Graf 2007).

An important consideration is that while the results from this study could be used to suggest dietary trends in conjunction with other data, the sample size is too small to detect the full range of dietary elements at BER. At least 15-20 samples are needed to detect 80-90% of major dietary components (Reinhard and Bryant 1992), so results can be considered a subset of the total diet. The absence of certain taxa in a cultural component cannot be taken to mean that they were not utilized. In addition, depending on whether traditional or molecular methods of defecator identification are used, the sample size of human-associated coprolites could be even smaller. Because of this, the data in this thesis alone are not suitable for determining the complete diet of residents at BER in a given cultural component but can show evidence for utilized foraging strategies. Regardless of the dietary resolution, the comparative analysis of genetic and

macroremains can be used to indicate differential preservation of materials, detect a larger number of taxa, compare defecator identification methods, and show how the chosen method could affect interpretations of sample contents.

Table 1 Provenience of Samples from Bonneville Estates Rockshelter.

Sample	Acc. #	Unit	Quad	Stratum	Provenience	Elevation
BiG4	11-4893-11243	N1W15	SE	3a	-	11-6 cm AD
BiG5	11-4893-5568	N3W4	-	3a	N84W10	26 cm BD
BiG6	11-4892-8771	N5W13	NE	3b	N5.71, W13.21	19 cm AD
BiG7	-	N4.5W16	SE	3b	N4.545, W16.14	30 cm AD
BiG8	11-4893-7964	N5W11	NE	3b	N5.76, W11.25	6 cm BD
BiG9	11-4893-24258	N7W22	SW	14a	-	50-46 cm BD
BiG10	11-4893-3605	26EK3682	SW	14a	N5.3, W15.89	39 cm BD
BiG11	11-4893-2516	N6W15	-	14a	N6.06, W15.67	20 cm BD
BiG12	11-4893-19534	N6W16	NE	14	N6.53, W16.32	31 cm BD
BiG13	11-4893-6693	N4W15	NE	14c	N4.72, W15.27	50 cm BD

Note: AD = above datum; BD = below datum, Acc. # = Accession number

Bonneville Estates Rockshelter and the Floristic Landscape

BER is situated in North America's eastern Great Basin where even minor changes in climate led to major alterations in the local biotic environment (Goebel et al. 2021; Grayson 2011; Louderback and Rhode 2009; Madsen 2001; Rhode and Madsen 1995). The rockshelter contains well-dated strata that span eight components dating from

Pre-Clovis through Late Prehistoric (~14,500 to ~500 cal yr BP) periods of occupation (Goebel et al. 2007, 2021; Hockett 2015), and the aridity of the site and region provides ideal conditions for fecal preservation (Goebel et al. 2021; see also Reinhard and Bryant 2002; Shillito et al. 2020). The Great Basin's environmental history is thoroughly documented, including data on both biotic and abiotic shifts from the Ice Age through the Holocene (Grayson 2011). Studies have also been done on the specific environmental and dietary history of BER and the broader Bonneville Basin (Hockett 2005, 2015; Madsen 2000; Schmitt and Lupo 2012). This is a site and region that has well-preserved samples, has clear environmental context, is well-dated, and has been the subject of multiple dietary studies that can be used as a comparative data source.

The dietary choices made by inhabitants of BER would have been greatly affected by the distribution of resources. Eastern Great Basin floral composition shifts significantly with elevation. At the lowest elevation is the playa, containing a variety of halophytic plants such as pickleweed (*Allenrolfea occidentalis*) while marsh resources and waterfowl can be found at the margins. Next is the valley floor, composed of desert shrubs such as shadscale (*Atriplex* sp.), greasewood (*Sarcobatus vermiculatus*), and saltbush (Amaranthaceae), cacti, other desert flora, and jackrabbits (*Lepus californicus*). As elevation increases into the lower foothills, floral communities contain shrubs like sagebrush (*Artemisia* sp.), horsebrush (*Tetradymia* sp.), and rabbitbrush (*Chrysothamnus* sp.), along with grasses including wild rye (*Elymus* sp.), wheatgrass (*Agropyron* sp.), and bluegrass (*Poa* sp.). The lower mountain slopes contain pinyon-juniper woodlands, while at higher elevations juniper (*Juniperus* sp.) gives way to mountain mahogany

(*Cercocarpus ledifolius*) and a recurrence of the sagebrush-grass zone with added *Ephedra*. At the highest elevations are subalpine forests, dominated by xeric conifers including Douglas fir (*Pseudotsuga menziesii*), white fir (*Abies concolor*), and limber pine (*Pinus flexilis*) (Goebel et al. 2021; Kelly 1997; Madsen 2000, 2007; Rhode 2008). Herbaceous resources could also be found in mountainous zones including wheatgrass, redtop grass (*Agrostis* sp.), bluegrass, needlegrass (*Stipa* sp.), gooseberry (*Ribes* sp.), currant (*Ribes* sp.), elderberry (*Sambucus* sp.), and other fruits (Chamberlin 1911, Goebel et al. 2021, Madsen 2000, Steward 1938). BER itself straddles two of these zones, meaning foragers would have been close to both the more upland sagebrush and more lowland shadscale communities (Goebel et al. 2021). They would also have had access to sage-grouse (*Centrocercus urophasianus*) and artiodactyls at higher elevations that may be out of reach of inhabitants of other sites (Chamberlin 1911; Goebel et al. 2021). It would be expected that inhabitants of BER would utilize resources from both these zones, with shifts depending on environmental context.

The Middle Holocene

The Middle Holocene (~8000-4000 cal yr BP) in the Great Basin is characterized by an overall increase in temperatures and a decrease in effective precipitation, leading to a more arid landscape (Goebel et al. 2021; Grayson 2011, Hockett 2007, 2015; Louderback et al. 2010; Schmitt and Lupo 2016). This was accompanied by a decrease in the wetlands and their associated resources that were more common and widely available during the Early Holocene. This shift to a more xeric environment caused

biotic shifts, as local flora became dominated by more desert-adapted plants in the lowlands as wetland plants were either constrained to small, well-watered lowland areas or retreated upslope into the mountains (Rhode 2008). Regionally, there was a general decrease in sagebrush and an increase in shadscale; additionally, the pollen record contains large amounts of cheno-ams, most likely *Atriplex* (Grayson 2011). The more mesic-adapted fauna present in the Early Holocene likely left the region or saw population decreases (Hockett 2007). Various mesic spikes occurred which corresponded to reoccupation of BER and re-abandonment as precipitation decreased again (Goebel et al. 2021; Hockett 2007).

Desertification was accompanied by an increase in small-seed processing, partially seen in the increase in grinding stone technology (Grayson 2011) and the associated processes of collection, grinding, winnowing, and parching necessary to make them ready for use. While small-seed use became more common, foragers preferentially utilized wetland resources when available (Rhode 2008). Another consideration is the utilization of faunal species during the Middle Holocene. This time frame saw a shift from rabbit (*Sylvilagus* sp.) to hare (*Lepus* sp.) utilization in the eastern Great Basin, as well as a decrease in the use of a variety of mesic fauna. Available fauna include pronghorn (*Antilocapra americana*), mountain sheep (Ovis canadensis), deer (*Odocoileus hemionus*), bison (*Bison bison*), hare, rabbit, sage grouse, bobcat (*Lynx rufus*), badger (*Taxidea taxus*), and weasel (*Mustela* sp.). Artiodactyl hunting also started to intensify, suggesting a large reliance on them for subsistence (Hockett 2015).

Component V

Component V encompasses strata 17a-12 and dates to between 8257 ± 50 and 4792 ± 70 cal yr BP (Table 2). Strata in this component are associated with the Early Archaic, and the defining cultural elements are large side-notched bifacial points and ground stone technology (Goebel et al. 2021). Site usage suggests an overall intensification of use of BER, with peak usage represented by Stratum 14 followed by a decrease in use. Stratum 14 contains multiple hearth features and large faunal deposits, and it shows evidence of the return of sagebrush to the shelter (Goebel et al. 2021).

The Late Holocene

The Late Holocene (~4000-150 cal yr BP) is characterized by periodic fluctuations between colder and warmer conditions with an overall shift from the warmer, drier Middle Holocene landscape to a cooler, wetter one (Hockett 2015; Janetski 1997; Madsen 2000, 2001; Schmitt and Lupo 2016). These climatic shifts resulted in changes in the floristic environment; sagebrush and conifer communities expanded during cooler periods while the number of cheno-ams decreased. Pinyon woodlands benefited from the relatively less arid landscape, spreading across lower mountain slopes (Louderback and Rhode 2009). The increase in effective precipitation also correlated to the expansion of grasses and the return of various highly-abundant wetland resources as wetlands and lakes re-appeared (Grayson 2011; Hockett 2005, 2015, Kelly 1997). More mesic-adapted fauna, such as bison, other large artiodactyls,

and rodents such as bushy-tailed woodrat (*Neotoma cinerea*), sage vole (*Lemmiscus curtatus*), and western harvest mouse (*Reithrodontomys megalotis*), which rely on grasses for food and habitat, returned to the region or increased their populations and ranges (Hockett 2005, 2015; Madsen 2000; Schmitt and Lupo 2016). Humans also returned and increased in numbers, shown by the high number of cultural features found dating to the Late Holocene, at Bonneville Estates Rockshelter and regionally (Goebel et al. 2021; Grayson 2011; Kelly 1997). At BER, human occupants at the site increasingly relied on artiodactyl species such as pronghorn, with additional use of mountain sheep, deer, bison, hare, rabbit, sage-grouse, bobcat, badger, and weasel (Hockett 2015).

Component III

Component III includes strata 10-3b and dates to between 4005 ± 65 and 1418 ± 53 cal yr BP (Table 2). It is associated with the Middle Archaic, with Elko corner-notched series points as the major diagnostic artifact. The component had fluctuating occupation rates, but the stratum in this study, 3b, contained the largest number of cultural remains (Goebel et al. 2021).

Component II

Component II is found in strata 3a and 2, and dates to between 1405 ± 52 and 856 ± 71 cal yr BP (Table 2). This component represents a relatively short phase that is associated with intense human occupation in the Late Archaic. It contains many hearth

and pit features and is differentiated from Component III by the appearance of bow and arrow technology (Goebel et al. 2021).

Table 2 Cultural Components at Bonneville Estates Rockshelter.

Component	Phase	Strata	Cultural Period	Age Estimates (cal yr BP)
I	Eagle Rock	2-1	Late Prehistoric	481 ± 50 to 130 ± 76
II	Maggie Creek	3a-2	Late Archaic	1405 ± 52 to 856 ± 71
III	James Creek	10-3b	Middle Archaic	4005 ± 65 to 1418 ± 53
IV	South Fork	11	Transitional	4717 ± 86 to 4156 ± 75
V	Pie Creek	17a-12	Early Archaic	8257 ± 50 to 4792 ± 70
VI	Wendover	17b	Earliest Archaic	10,021 ± 105 to 8581 ± 53
VII	Dry Gulch	18b-17b’	Paleoindian	12,941 ± 71 to 10,531 ± 82
VIII	Pre-Clovis	20-19	Pre-Clovis	14,516 ± 182 to 13,397 ± 45

Data from Goebel et al. (2021).

From Metadata to Sequencing

Metadata including physical descriptions, measurements, and photographs were collected for each sample using the methods described by Juoy-Avantin et al. (2003) (Table 3).

Table 3. Coprolite Metadata

	Color	Volume	Extremity 1	Length	Min Width	Max Width	Taph. Mod.
4	5yr3/3	sp	RO	25.4	19.84	21.43	GA
5	2.5y7/1	CY	BR	31.75	19.05	25.4	AB
6	7.5yr3/2	cy	BR	31.75	19.05	25.4	AB
7	2.5y4/2	Un	BR	58.65	27.46	44.12	Ab
8	10yr8/2	cy	BR	12.7	15.88	15.88	AB
9	10yr5/4	FL	BR	34.93	26.99	34.93	GA
10	10yr8/2	FL	BR	57.15	25.4	31.75	FI
11	10yr5/4	CY	BR	38.1	12.7	19.05	AB
12	10yr5/3	CY	RO	55.56	19.05	19.05	AB
13	7.5yr3/3	sp	BR	24.93	23.02	33.34	FI
	Pres.	**Constrictions**	**Extremity 2**	**Min Thickness**	**Max Thickness**	**Weight**	**Inclusions**
4	E1	0	RO	12.7	12.7	2.00	AB
5	F1	0	RO	6.35	15.88	7.00	PF
6	E2	0	RO	12.7	19.05	6.00	PF
7	F1	0	BR	22.54	27.06	5.38	ST, PF
8	F1	0	BR	3.175	15.08	1.00	PF
9	**F1**	0	BR	5.52	14.29	4.00	SC, PF
10	**E2**	0	BR	7.94	12.7	16.00	PF
11	**F1**	0	RO	11.91	19.05	6.00	FE, PF
12	**F1**	1	BR	15.08	19.05	11.34	PF
13	F2	0	BR	12.7	19.05	21.54	PF

Length, width, and thickness in (mm); weight in (g); Taph. Mod = taphonomic modifications, Pres. = preservation

Key: E1: entire, F1: isolated fragment, Ex: restored with x fragments, Fx: cannot be restored with x fragments, sp: spherical by extrapolation, CY: cylindrical, cy: cylindrical by extrapolation, UN: undetermined, FL: flat, RO: round, BR: broken, GA: gallery-hole, AB: absence, FI: fissure, PF: plant fiber, ST: stones, SC: shells, FE: feathers

Subsampling was performed in the Bioarchaeology and Genomics (BiG) Laboratory at Texas A&M University using the protocol in Wood and Wilmshurst (2016). In brief, this involved UV irradiation and removal of the exterior, UV irradiation of the new surface, and collection of two 250 mg samples from the interior. All samples were lightly ground in their collection tubes, after which DNA was extracted in the BiG Lab using the DNeasy PowerSoil Kit with standard protocol (Qiaqen). This involved

initially adding samples and Solution C1 to a PowerBead Tube containing garnet beads and a lysis buffer to break down the samples. Samples were vortexed and centrifuged, after which Solution C2 was added to precipitate non-DNA material. Samples were spun and the supernatant added to Solution C3 for inhibitor removal. After centrifugation, the supernatant was added to Solution C4, loaded onto an MB Spin Column, and spun. This allowed for the DNA to bind to the silica in the column. Solution C5 was then added directly to the column; the samples were spun to further wash the bound DNA. One sample from each coprolite was left bound in silica while the other was fully eluted in Solution C6, an elution buffer. This resulted in two DNA samples per coprolite: one bound and one eluted. Each preparation additionally had an associated negative control.

Samples were then transported to the Trace and Environmental DNA (TrEnD) Laboratory at Curtin University for metabarcoding, library construction, and sequencing. The bound samples were first eluted from the silica membrane in buffer EB (Qiagen), after which all samples were prepped for DNA metabarcoding. Metabarcoding utilizes universal primers to amplify a target gene; the base primers in this study are trnL, which targets the plant chloroplast trnL intron, and 12sv5, which targets the vertebrate mitochondrial 12s rRNA gene (Table 4) (Pederson et al. 2015; Staats et al. 2016). An initial amplification using these base primers was performed on neat and 1:10 dilutions of each sample, along with negative controls, with the following reaction mixture: 2 µL DNA extract, 2.5 µL 1x Gold PCR buffer, 2.0 µL $MgCl_2$, 0.25 µL 25 mM dNTPs, 0.5 µL 10 mM forward and reverse primers, 0.25 µL AmpliTaq Gold DNA polymerase, 0.6 uL SYBR Green, 1.0 µL bovine serum albumin, and enough ultrapure water to reach a final

volume of 25 uL. PCR cycling started with a 5-minute denaturation at 95° C, followed by 40 cycles of 30 seconds at 30° C, 30 seconds at 57° C or 52° C depending on the primer (Table 4), and extension at 72° C for 2 minutes. This was followed by a final extension of 10 minutes at 72° C.

Table 4 Primers

Primer	Forward Primer	Reverse Primer	Annealing Temp
12SV5	TAGAACAGGCTCCTCTAG	TTAGATACCCCACTATGC	57°C
trnL-gh	GGGCAATCCTGAGCCAA	CCATTGAGTCTCTGCACCTATC	52°C

The cycle thresholds, or Ct, values were used to determine which dilution resulted in greater amounts of the target amplicons, where an amplicon is the product of PCR amplification. Ct values represent the point in PCR cycling where detectable amounts of the amplicon have been generated. This means lower Ct values correlate to greater amounts of target product. The dilutions with lower Ct values were chosen for each sample and each primer set for metabarcoding. Each sample, along with negative controls, was assigned a unique set of forward and reverse metabarcoding primers; these are composed of the base primers (Table 4) and extra base pairs called tags. Assigning samples unique tag combinations allows for their identification post-sequencing. The tagged, metabarcoded amplification was performed on the chosen samples and negative controls using the same protocol as described for the untagged amplification.

Samples with undetermined Ct values were removed, and the rest were organized into a total of ten minipools based on similar Ct values ranging from 21-41. Each minipool contained between four and seven samples and was made by adding 10 uL of each tagged, amplified sample to the corresponding collection tube. Each minipool was

cleaned with a QIAquick PCR Purification Kit (Qiagen) using the standard protocol with variations; samples were washed with 650 uL buffer PE and eluted with 30 uL buffer EB. Minipools were quantified on Qiaxcel, after which equimolar amounts of each minipool were combined in a single library to a final volume of 66 uL. The library was split into two 30 uL fractions and 10 uL of Pippin Prep loading solution was added to each. Both library fractions were size selected on a Pippin prep to 160-450 bp, recombined, and cleaned using the same protocol as described above. Sequencing was done to 1 million reads, each of which represents a DNA sequence, on single-end mode to 300 bp on the Illumina MiSeq platform.

Ancient DNA Downstream Analysis

Reads were first input into Geneious v11.1.5 and sequencing adapters were trimmed. Reads were then sorted based on their tags; reads with both a forward and reverse tag were attributed to individual coprolite samples while reads with either one or no tags were removed from analysis as they could not be linked to a particular sample. The primers and tags were then trimmed from the reads, resulting in a collection of DNA sequences that were linked to specific samples and only contained the target gene. Reads belonging to the same sample and same base primer set were grouped and dereplicated, which is when identical reads are grouped. This was done using the USEARCH pipeline as described by Murray et al. (2013). Chimeric reads, sequences that are hybrids of multiple different reads, and singletons, sequences represented by a single read, were also removed. Sequences represented by two or more reads were kept in the dataset.

Reads were then grouped into operational taxonomic units, or OTUs, in USEARCH using the UPARSE-OTU algorithm (Edgar 2013).

OTUs are made by grouping highly similar reads, in this case those with 97% similarity, to act as a proxy for an organism outside of taxonomic classification and resulting in a concise list of DNA sequences representing the likely genetic diversity of the sample. Because coprolites represent multiple organisms, it is expected the total reads from one sample will be grouped into multiple OTUs. The most abundant read in each OTU is used to represent the group as a whole, and the number of reads contained in that OTU is recorded (Edgar 2013). OTUs can then be ordered in terms of abundance; OTUs with higher numbers of reads are considered more abundant in a sample while those with lower numbers are considered less abundant. Low abundant groups, those that represent less than 1% of all unique sequences, were removed. As described by Murray et al. (2013), the removal of low abundant groups reduces the amount of noise attributable to sequencing errors.

The representative of each OTU was then aligned to reference DNA sequences in the NCBI nr database (accessed July 30, 2018) using BLASTn v2.7.1, assigning them a taxonomic identification. The results were visualized in MEtaGenome Analyzer v6 (MEGAN), using the LCA (lowest common ancestor) assignment algorithm with a min score of 65, min support of 1, and top % of 5 to analyze the taxonomic content of individual samples (Huson et al. 2016). Read numbers and their associated taxa were compiled for each coprolite at the family level, and to lower taxonomic levels when

possible. Samples containing human DNA and little to no dog DNA were considered human coprolites, while samples containing dog DNA with little to no human DNA were considered dog coprolites. Samples with no human or dog DNA were considered as having an unknown source.

Samples were considered both individually and more broadly by stratum (or component). Taxonomic abundance in individual samples was determined by percent composition according to read number, while ubiquity across components and the overall assemblage was determined by counting how many samples contained a certain taxon. To visualize the composition of individual coprolites, read counts for each OTU at the family level were loaded into R v3.6.3. OTUs that were not assigned taxa or had no hits were kept in the dataset. Reads in these OTUs passed all quality checks and filters, were successfully grouped into OTUs, and represent greater than 1% of all unique sequences. Their exclusion would result in a skewed dataset, as the unassigned OTUs range in abundance across a subset of the samples. Reads attributed to organisms that are not native to the region or were not present at the time of sample deposition were merged in individual samples and labelled as contamination. The counts were converted into percent and plotted to look at broad differences between coprolites from the different components. In cases where taxa were only identified to the family level, ethnographic and environmental data was used to suggest, though not assign, potential taxa. The overall contents of each component were then compared to see which taxa were shared between components or were unique to one.

Macroremains Analysis

After subsampling for DNA, half of each remaining sample was selected for macroremains analysis and processed according to the methods described by Fry (1976; 1985). Samples were weighed and placed in sealed containers with enough 0.5% trisodium phosphate to cover them. These were left in a cool, dark place and mixed at least twice a day for a duration of up to two weeks for disaggregation, after which the color and smell of the liquid was recorded. Samples that had not broken down at this point were mechanically disaggregated. Samples were screened through 250-micron mesh; the liquid portion was saved while the solid portion was placed in petri dishes and air dried. Once dry, additional mechanical disaggregation was performed on samples with high fiber and hair content. Samples were then dry-screened through an 850 and 250-micron mesh. This resulted in at least two fractions for all samples but one.

The < 250-micron fraction was left unsorted while the 850- and 250-micron fractions were viewed through a dissecting scope under 10-15x magnification. The 250-micron portion was qualitatively analyzed to determine its components, while the 850-micron fraction was fully sorted into floral and faunal components. This fraction was further separated into individual floral taxa categorized by shared morphological traits, unidentified floral remains including fibers, fragments, epidermis, and leaf-like material, faunal remains including bone, hair, eggshell, feather, and insect parts, and inorganic materials including stone and charcoal. Additional material from the 850-micron fractions that could not be separated without possibly damaging the samples were

labelled as miscellaneous, and their general composition was recorded. Materials from the 850-micron fraction were weighed and the number of individual specimens (NISP) counted for identified remains. Identifications were made through comparison to illustrated databases of seeds in the region in addition to direct comparison with the comparative collection housed in the Paleoethnobotany Laboratory at Texas A&M University. Taxa were identified to at least the family level with most identified to genus or species. Ubiquity was measured according to the number of samples containing a certain trace while abundance was determined by converting weights to percent. Component contents were then compared to detect shared and unique taxa.

CHAPTER III

RESULTS

Ancient DNA

Seventeen taxa were identified to the family level with additional reads either not assigned or garnering no hits (Table 5). Human DNA was detected samples 7, 8, 9, 10, and 11; these were labelled as having a human source. Dog (*Canis familiaris*) was found in two samples; one of these, sample 5, contained large amounts of dog DNA with no human DNA and was labelled as having a canine depositor. The other, as is shown below, had significantly more human DNA and was labelled human. Pronghorn and black-tailed jackrabbit (*Lepus californicus*) were both found in one sample each. Samples 4, 6, 12, and 13 contained no faunal reads and were considered as having unknown depositors. The remaining identified taxa were floral. The most ubiquitous was Chenopodiaceae, found in five samples. This was followed by Pinaceae, which was found in four samples, and Asteraceae, detected in three. Apiaceae, Poaceae, Cupressaceae, and Ephedraceae were all found in two samples, while Boraginaceae, Proteaceae, Rosaceae, Euphoribaceae, Solanaceae, and Brassicaceae were each found in one. Unassigned reads were found in seven samples, while nine contained reads with no hits (Table 5, Table A2).

Table 5 Taxa Identified by DNA Metabarcoding in Components V, III, and II

Family	Lowest Taxonomic Level	Common Name	V (n=5)	III (n=3)	II (n=2)
Hominidae	*Homo sapiens*	Human	3	2	-
Antilocapridae	*Antilocapra americana*	Pronghorn	-	1	-
Canidae	*Canis familiaris*	Domestic Dog	-	1	1
Leporidae	*Lepus californicus*	Black-Tailed Jack Rabbit	1	-	-
Apiaceae	Apioid Superclade	Carrot Family	1	-	1
	Acronema Clade		1	-	-
Asteraceae		Daisy Family	1	1	-
	Asteroideae		-	1	-
	Artemisiinae		-	2	-
	Anthemideae		-	1	-
Boraginaceae	*Lappula*	Stickseed	-	-	1
Brassicaceae		Mustard Family	1	-	-
Chenopodiaceae	*Atriplex*	Saltbush	1	3	1
	Corispermum	Bugseed	-	1	-
Cupressaceae		Juniper	1	1	-
Ephedraceae	*Ephedra*	Mormon Tea	1	1	-
Euphoribaceae	***Hevea***	**Rubber Tree**	**-**	**-**	**1**
Pinaceae	*Abies*	Fir	-	2	-
	Pinus	Pine	1	1	-
	Pinus subgen		1	1	-
Poaceae	Poeae Chloroplast Group 2	Grasses	-	1	-
	Stipeae		-	1	-
	Triticodae		-	1	-
	PACMAD Clade		-	1	-
	Cenchrinae		-	1	-
Proteaceae		**Protea Family**	**1**	**-**	**-**
Rosaceae	Dryadoideae	Rose Family	-	1	-
Solanaceae	*Nicotiana attenuate*	Tobacco	-	1	-
Other	No hits		4	3	2
	Not assigned		3	3	1

Taxa in boldface are likely contamination.

Component V: Ancient DNA

Ten families were detected across the Component V coprolites (Figure 3). When considering the unknown samples, 12 had no identified reads. While the majority (92%) were not assigned, they most closely resemble cheno-ams, which are widely found in the region and are known food sources. Sample 13 contained similar unassigned reads but additionally contained reads attributed to Proteaceae. This is likely contamination, as the family contains tropical plants endemic to Western Australia where library prep and sequencing were conducted.

Approximately half of all reads in samples 9, 10, and 11 were attributed to human, with the rest of the identified reads belonging to a total of eight taxa (Figure 3). Sample 9 displayed the widest dietary breadth of this component and contained the only non-human faunal trace found in the Component V samples. Black-tailed jackrabbit was the most abundant, comprising 17% of total reads while the other taxa comprised less than 1% combined. In order of abundance, these are Chenopodiaceae, specifically *Atriplex*, Pinaceae, specifically *Pinus*, Cupressaceae, Brassicaceae, and Ephedraceae, specifically *Ephedra*. Sample 10 contained significantly less diversity; Asteraceae was the only non-human taxon detected. The dietary elements detected in sample 11 were composed of 37% Apiaceae and 9% *Pinus*. As with the unidentified samples, unassigned reads in the possible human samples are closest to cheno-ams.

Component III: Ancient DNA

Ten families were identified in Component III coprolites (Figure 3). Sample 6, which has an unknown defecator, was approximately 50% *Atriplex* with no other identified reads. Samples 7 and 8 contained 28% and 9% human DNA, respectively, and display similar dietary breadth. Sample 7 contained a total of six taxa, with the largest component being Poaceae at 28%. This was followed by 13% Chenopodiaceae; the majority of these reads were attributed to *Atriplex*, with less than 1% attributed to *Corispermum*. Approximately 9% of reads were Rosaceae, 3% were Asteraceae, 2% were *Abies,* and less than 1% were attributed to Solanaceae, specifically *Nicotiana attenuata*.

Of the nine identified taxa, sample 8 contained two non-human faunal traces. These were 15% pronghorn and < 1% dog. The major floral component was Pinaceae, which was divided into *Pinus* (23%) and *Abies* (7%). This is followed by 9% *Atriplex*, 6% Cupressaceae, 6% Poaceae, 4% *Ephedra*, and 1% Asteraceae. As with the Component V samples, unassigned reads in all samples resembled cheno-ams, with the exception of those of sample 8 which more closely resembled *Ephedra*.

Component II: Ancient DNA

Five taxa were identified in Component II samples, but neither showed traces of human DNA (Figure 3). Sample 4 was predominantly (50%) Boraginaceae, specifically *Lappula*. Smaller amounts (< 1%) of Apiaceae and *Atriplex* were also detected. Sample 5, the possible dog coprolite, contained only two identified taxa. Approximately 60% of

all reads were dog, while < 1% were from Euphoribaceae, specifically *Hevea*. This was considered contamination as it is a rubber tree and not native to the region. Unassigned reads resembled cheno-ams.

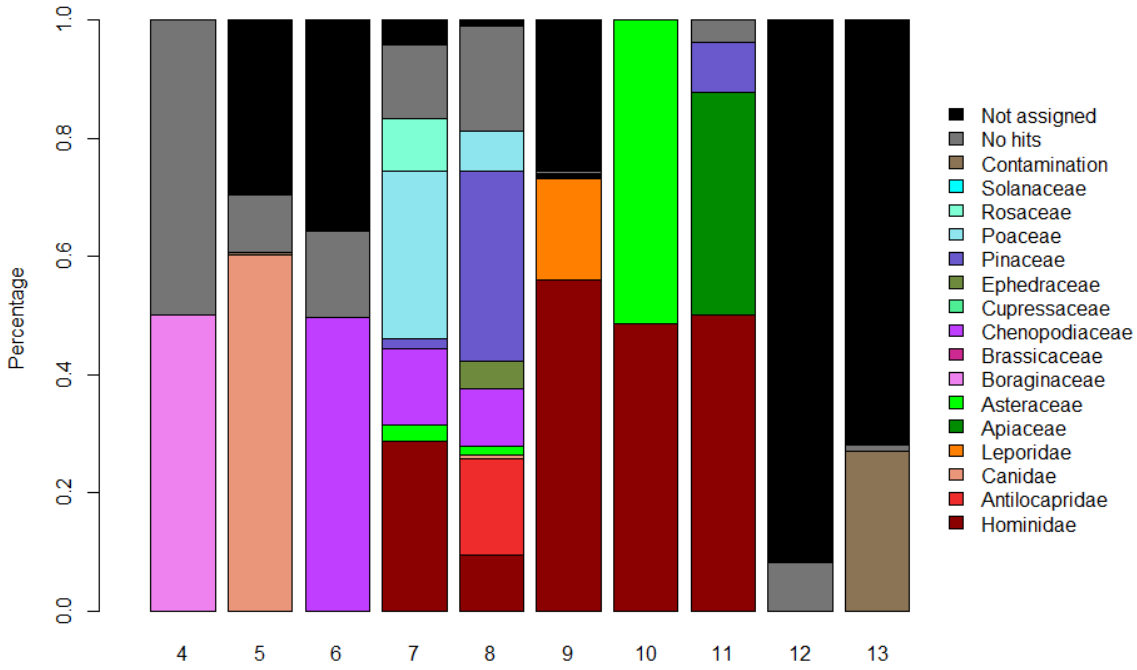

Figure 3 Family-level taxonomic composition of coprolites based on genetic data.

Shared and Unique Taxa Between Components: Ancient DNA

Samples from Component V contained two unique taxa, Brassicaceae and black-tailed jackrabbit (Figure 4). Brassicaceae, known as the mustard family, is composed of annual and perennial herbs and cruciferous vegetables. Component III contained a larger number of unique taxa, containing pronghorn, Poaceae, Rosaceae, and Solanaceae (Figure 4). Poaceae, a family containing a variety of grass species, had reads identified at lower taxonomic levels as Poeae Chloroplast Group 2, Stipeae, Triticodae, the PACMAD Clade, and Cenchrinae. No reads were identified to the genus or species.

Reads attributed to Rosaceae were more specifically identified as Dryadoideae, while the Solanaceae component was the only taxon identified to the species level as *Nicotiana attenuate*. Genera unique to Component III samples are *Corispermum* and *Abies*. The former is part of Chenopodiaceae and more commonly known as bugseed, while the latter is part of Pinaceae and includes a variety of firs. The only taxon unique to Component II is Boraginaceae (Figure 4). Known as the borage family, it contains small perennials, herbs, shrubs, and various wildflowers. The identified genus, *Lappula*, is commonly known as stickseed.

Nine taxa were shared between at least two components. Only *Atriplex*, known as saltbush or shadscale, was detected in all three. Components III and V share four taxa other than human: Asteraceae, or the daisy family, Cupressaceae, the conifer family, *Ephedra*, and *Pinus*. Component II only contained two shared taxa; both II and III contained dog DNA while II and V contained reads from Apiaceae (Figure 4).

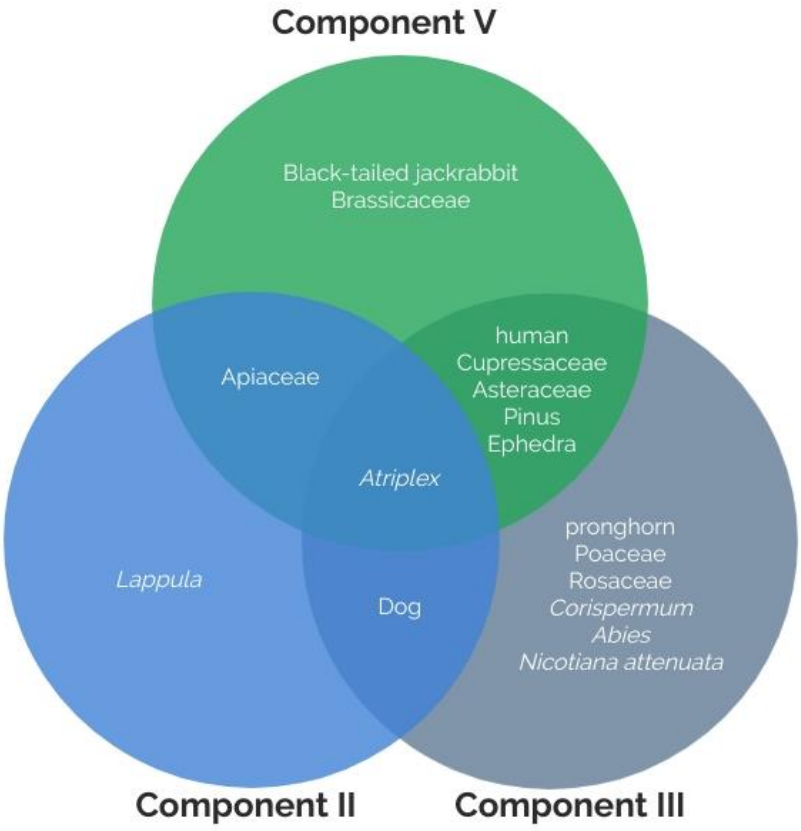

Figure 4 Shared and unique taxa between components II, III, and V based on genetic data.

Macroremains

Nineteen kinds of macroremains were found in the BER coprolites across all three components (Table 6). Six of these were identifiable to at least the genus or species level with an additional taxon identified to the family level. An additional four kinds of plant remains, five kinds of faunal remains, and two kinds of inorganic remains were also detected across the samples. The most ubiquitous of these are seeds of *Allenrolfea occidentalis*, or pickleweed, and bone; both were found in seven samples. Undifferentiated plant fibers, feathers, stone, and charcoal were each found in four

samples, while plant fragments, eggshell, and hair were found in three. Only two samples contained leaf-like material and insect remains, while the rest of the materials were only found in one each. These are unidentified plant epidermis, pigweed (*Amaranthus* sp.), *Chenopodium* cf. *nevadense* (goosefoot), *Acnatherum hymenoides* (ricegrass), *Atriplex, Artemisia*, cf. Poaceae, and cf. *Opuntia*.

Table 6 Ubiquity of Macroremains in Components V, III, and II.

		V (n=5)	III (n=3)	II (n=2)
Identified Flora	*Allenrolfea occidentalis*	4	1	2
	Amaranthus	1	0	0
	Chenopodium cf nevadense	1	0	0
	Acnatherum hymenoides	0	1	0
	Atriplex	0	1	0
	Artemisia	0	1	0
	Cf Poaceae	0	0	1
	Cf Opuntia	0	1	0
Bulk Flora	Plant Fiber	2	2	0
	Plant Fragments	1	2	0
	Plant Epidermis	0	0	1
	Leaf-Like	1	1	0
Fauna	Bone	3	2	2
	Eggshell	1	1	1
	Hair	2	1	0
	Feather	2	1	1
	Insect	0	1	1
Inorganic	Charcoal	2	1	1
	Stone	2	1	1

The defecator was determined using traditional methods, as described in Section 1.2.1. Preliminary identifications were made by analyzing the color of the rehydration liquid. Samples 4, 6, 7, 10, 11, and 12 turned dark and opaque, suggesting a human origin, while samples 5, 8, 9, and 13 turned the liquid a lighter, translucent color,

suggesting a non-human origin (Table 7). However, as not all coprolites follow this trend, their contents were used to make further assumptions about their source during analysis.

Table 7 Rehydration Characteristics

	Subsample Weight (g)	Liquid Color	Translucent	Smell	Scum
4	0.80	7.5yr2.5/2	No	Med Musty	No
5	3.22	2.5yr7/8	Yes	Light Fecal	No
6	1.31	5yr2.5/2	No	Light Musty	No
7	4.03	5yr2.5/2	No	Med Musty	No
8	0.58	2.5yr7/8	Yes	Med Fecal	No
9	1.62	7.5yr4/6	Yes	Med Musty	No
10	5.26	5yr2.5/2	No	Med Musty	No
11	2.3	7.5yr3/2	No	Med Fecal	No
12	5.42	5yr2.5/2	No	Med Musty	No
13	3.94	7.5yr3/2	Yes	Light Fecal	Yes

Component V: Macroremains

Twelve distinct macroremains were found in the Component V coprolites; three were identified to the genus or species level while the rest were undifferentiated or unidentified. Overall, the floral assemblage is dominated by plant fiber and small seeds, while the faunal assemblage is dominated by bone. Initial analysis was done on samples 9 and 13 to see if their contents suggest a human defecator even though the rehydration liquid did not. The most abundant material by weight (70%) in sample 9 is plant fiber, followed by about 10% of a leaf-like material and almost 20% bone (Table 8). Trace amounts of hair and feathers are present, as are one pickleweed seed and one piece of charcoal. Even though sample 9 did not turn the rehydration liquid dark, the contents could be indicative of a human sample; it contains both floral and faunal elements suggesting omnivory, and the presence of charcoal suggests the consumption of cooked

food. Sample 9 had no 250-micron fraction. Sample 13 contained significantly less material, as it is almost entirely composed of fragmented pickleweed seeds in both the small and large fractions, with very little bone and fiber, and a single stone. However, because of the size of the faunal and inorganic elements, they account for approximately 11% of the total weight, while pickleweed accounts for 89% (Table 8). The integration of seeds with the surrounding coprolite matrix made their separation largely infeasible without damaging the samples, but it is possible that this sample is also human.

Sample 10, identified as likely human, contained six types of macroremains. The most abundant by weight is miscellaneous; 59% of the sample is composed of integrated fiber, small stones, and fragmented cheno-ams (Table 8). Three kinds of cheno-ams were identified to at least the genus level as pickleweed, *Amaranthus*, and *Chenopodium* cf. *nevadense*, or goosefoot. The next most abundant was bone at 41%. Trace amounts of hair, charcoal, and stone were also found, and the 250-micron fraction was composed of fragmented cheno-ams. Sample 11 contained four kinds of remains, none of which was identified. The most abundant (77%) is a feather-eggshell mixture, while the other 23% is made up of eggshell alone. Trace amounts of individual feathers and plant fragments were also found, and the 250-micron fraction contains primarily plant fragments. The final probable human sample, 12, is composed of 94% plant fiber, while the other 6% is from a piece of cordage that appears to be made of the same fiber found in this sample and others (Table 8). Pickleweed seeds were found in the fiber and the cordage, and the 250-micron portion contains plant fibers, additional fragmented pickleweed seeds, and charcoal. Based on contents, all samples from Component V could be human.

Component III: Macroremains

Fifteen kinds of remains were in the Component III samples. Five were identified to at least the genus level, while the other ten remained unidentified. The most ubiquitous remains are plant fiber, plant fragments, and bone. Sample 8, the Component III coprolite with unknown origin, is composed of a homogeneous plant fiber (Table 8). The fiber is so dense that the sample could not be fully disaggregated, either through chemical or mechanical means.

Sample 6, identified as likely human, contains eight kinds of remains. The most abundant is plant fiber, accounting for the total weight. The only identified macrofossil was pickleweed, which represented the majority of non-fibrous remains. This was followed by plant fragments, a single fragment of a leaf-like material, and a piece of charcoal. Faunal remains include trace amounts of bone, eggshell, and feather. The 250-micron portion reflects this and is composed of fragmented pickleweed, plant fiber, plant fragments, and small amounts of feather, and the contents support a human depositor.

Sample 7 contains a total of ten distinct remains. The most abundant by weight was stone (84%), but their sizes and absence from the interior portions indicate they may have been pushed into the sample post-deposition. The next most abundant material is charcoal, accounting for 11%, followed by plant fragments at 5%. Trace amounts of bone and hair are present (Table 8). Of the identified remains, *Acnatherum hymenoides*, or Indian ricegrass, is most abundant, followed by near-equal amounts of *Atriplex*, *Artemisia*, and cf. *Opuntia* in the form of spines and a glochid. The 250-micron fraction

is primarily composed of Indian ricegrass seed and chaff fragments, charcoal, bone, and small stones. All Component III samples could be human.

Component II: Macroremains

Component II samples contained nine kinds of macroremains. One was identified to the species and another to the family; the remaining materials were unidentified. Sample 5, not identified as human according to rehydration liquid, contains eight kinds of material. Bone is most abundant at 68%, followed by 27% miscellaneous coprolite matrix composed of feather, insect remains, and bone fragments. While the bone assemblage is highly fragmented and lacks morphological significance, it looks overwhelmingly avian. The remaining 5% is eggshell (Table 8). Less-abundant materials include pickleweed, cf. Poaceae, unidentified plant epidermis, feathers, insect fragments, and charcoal. The 250-micron fraction contains a mixture of bone, feather, shell, pickleweed, insect, and grass remains. While the contents could be present in a human diet, the high amount of large bone fragments, along with the white color of the coprolite's exterior, suggest sample 5 has a canine origin.

Sample 4, identified as possibly human, is mostly composed of a homogeneous matrix of digested plant material. It contained a single pickleweed seed and bone fragment, and the 250-micron fraction contained trace amounts of charcoal and stone. The contents of sample 4 correlate with the rehydration liquid in identifying the sample as having a human source.

Table 8 MNI of Identified Specimens and Weights of Bulk Macroremains.

		4	5	6	7	8	9	10	11	12	13
Identified Flora	*Allenrolfea occidentalis*	T1	T16	T97			T1	T4		T13	T7
	Amaranthus							T6			
	Chenopodium cf nevadense							T10			
	Acnatherum hymenoides				T53						
	Atriplex				T3						
	Artemisia				T3						
	Cf Poaceae		T9								
	Cf Opuntia				T5						
Bulk Flora	Plant Fiber			0.18		0.45	0.58			1.6	
	Plant Fragments			0	0.07				0		
	Plant Epidermis		0								
	Leaf-Like			0			0.08				
Fauna	Bone	0	0.75	0	0		0.15	0.8			0.1
	Eggshell		0.06	0					0.2		
	Hair				0		0	0			
	Feather		0	0			0		0		
	Insect		0		0						
Inorganic	Charcoal		0	0	0.15		0	0			
	Stone	0			1.14			0			0.1
Misc.		0.12	0.3					1.1	0.8	0.1	1.1
Total		0.12	1.11	0.18	1.36	0.45	0.81	1.8	1.1	1.7	1.03

Weights are in grams; T denotes trace followed by minimum number individuals (MNI)

Shared and Unique Taxa Between Components: Macroremains

Samples from Component V contained two unique traces, *Amaranthus* and *Chenopodium* cf. *nevadense*, or goosefoot. Component III had the largest number of unique traces, including *Atriplex*, *Artemisia*, cf. *Opuntia*, and *Acnatherum hymenoide*s, or saltbush/shadscale, sagebrush, prickly pear, and Indian ricegrass, respectively. Component II had only two unique elements, one was identified as cf. Poaceae and the

other was unidentified plant epidermis (Figure 5). While outside the skill of the researcher, the epidermis is likely identifiable.

Six kinds of macroremains were shared between all three components: *Allenrolfea occidentalis*, or pickleweed, bone, eggshell, feather, stone, and charcoal. Of these, pickleweed is most ubiquitous in Component V samples, while the other remains are about equal across samples. Components III and V shared four kinds of remains, none of which was taxonomically identified. These are plant fiber, plant fragments, leaf-like material, and hair. Components II and III coprolites only shared insect remains, while there were no remains exclusive to just components II and V (Figure 5).

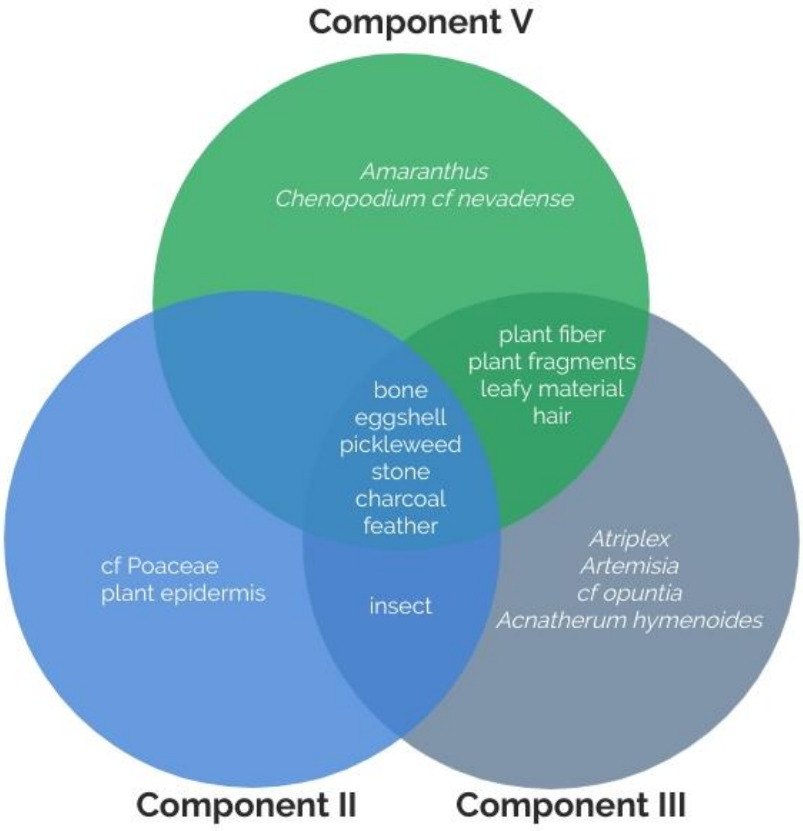

Figure 5. Comparison of contents of macroremains across components V, III, and II.

CHAPTER IV

COMPARATIVE ANALYSIS

The Genetic Data: Ethnographic Accounts

Many of the floral taxa identified in the genetic data contain members with known ethnographic uses. While not identified below the family level, ethnographically consumed Brassicaceae in the eastern Great Basin includes the seeds and leaves of plants such as hedge mustard (*Sisymbrium*) and watercress (*Nasturtium*) (Table A1; Chamberlin 1911; Steward 1938). However, these may not be endemic to North America (Welsh and Reveal 1977), meaning additional species need to be considered such as the ethnographically consumed and native desert prince's plume (*Stanleya pinnata*), entireleaved thelypody (*Thelypodium integrifolium*), tansy mustard (*Descirainia pinnata*), and heartleaf twistflower (*Stretanthus cordatus*) (Table A1; Rhode 2002). When looking at Poaceae, the seeds of plants belonging to the identified grass groups would have been consumed. Possibilities include reed grass (*Cinna*), blue grass (*Poa*), ricegrass (*Acnatherum*), brome grass (*Bromus*), wheat grass (*Agropyron*), and needle grass (*Stipa*) (Table A1; Chamberlin 1911; Steward 1938). Rosaceae contains a wide range of herbs, shrubs, and trees, but Dryadoideae only contains a total of four genera; *Cercocarpus*, *Purshia*, *Dryas*, and *Chamaebatia*, which significantly decreases the number of possible taxa. *Cercocarpus* includes mountain mahogany (*Cercocarpus ledifolius*), which was locally available and frequently used for making bows and fires, as well as used medicinally to treat burns (Table A1; Chamberlin 1911; Steward 1938; Rhode 2002). *Purshia*, most likely *P. tridentata*, is commonly known as antelope

bitterbrush and grows in sagebrush and pinyon-juniper woodland environments near the rockshelter. *Dryas* can be found in more alpine, mountainous regions of the Great Basin while *Chamaebatia foliolosa* (the only species in this genus) is endemic to California.

Reads attributed to *Nicotiana attenuata* represent a native, wild form of tobacco that would have been primarily smoked (Table A1; Chamberlin 1911; Steward 1938; Rhode 2002), and taxa from Boraginaceae have both dietary and medicinal uses. The consumption of *Atriplex* seeds is present in ethnographic accounts, and possible species include *A. confertifolia*, *A. truncata*, and *A. canescens* (Table A1; Chamberlin 1911; Steward 1938; Rhode 2002). Asteraceae, or the daisy family, is one of the largest floral families, with many dietary or medicinal taxa (Table A1). However, additional reads identified at lower taxonomic levels suggest some of these reads may be from *Artemisia*, or sagebrush. Sagebrush is in the Asteroideae subfamily, Anthemideae tribe, and Artemisiinae subtribe, all of which were identified in Component III samples. Sagebrush includes species such as *A. tridentata*, *A. biennis*, *A. discolor*, *A. trifida*, and *A. dracunculoides*. Sagebrush seeds are edible; accounts state the leaves were used to treat fevers and respiratory illnesses, and it was also used to make fire and cover food storage (Table A1; Chamberlin 1911; Steward 1938; Rhode 2002).

Cupressaceae includes junipers, and possible species detected in pollen records include *J. osteosperma*, *J. scopulorum*, and *J. communis* (Louderback and Rhode 2009). The berries may have been eaten, the leaves made into tea to treat colds, and the wood used for fire, construction, and to line storage pits (Table A1; Chamberlin 1911; Rhode 2002). Pinaceae, specifically *Pinus*, includes species such as *P. flexilis*, or limber pine,

and the highly important *P. monophylla*, or pinyon pine. Pinyon pine was one of the most important food resources available to inhabitants of the region and would have been gathered annually (Chamberlin 1911; Steward 1938; Rhode 2002). Cones were often partially charred, and the nuts eaten directly or ground (Chamberlin 1911, Steward 1938). Another ethnographically important family is Apiaceae, known as the carrot family. It includes edible plants such as biscuit root, yampah, and Indian potato, as well as cow parsnip and parsley. Edible parts include the leaves, shoots, and roots, while the roots of some species could be applied to wounds as a paste (Table A1; Chamberlin 1911; Steward 1938). The final floral taxa, *Ephedra*, is known as Mormon tea, jointfir, or joint-pine, and has medicinal uses (Chamberlin 1911; Steward 1938; Rhode 2002).

Of the two faunal taxa, black-tailed jackrabbit is ubiquitous in the region and would have served as an important source of food and skins (Steward 1938). Ethnographic accounts state they were often communally hunted by driving them out of the brush and into awaiting nets and traps (Steward 1938). Pronghorn would have been less important than plants and smaller game in subsistence but was hunted communally (Steward 1938).

Conclusions: The Genetic Data

The first consideration when looking at the genetic data is defecator identification. While only five samples contained human DNA and were labelled as having human sources, this may not be accurate. It is possible that other samples also contain human DNA that would be detectible when using a different amplicon, a

targeted PCR approach, or through shotgun sequencing. The reads found in samples with no human DNA could relate to known dietary elements, so the number of human coprolites may be higher than suggested above. Sample 5, however, contains such a large amount of dog DNA that it is likely dog. Also, even if the coprolites with unknown and dog defecators did not come from humans, their contents still would have been available to the inhabitants of BER.

Coprolites from Component III display the largest dietary breadth in this data set, followed by those from Component V. Component II samples displayed the smallest diet breadth of the three. However, as was stated in Chapter 2 the sample size of ten is not large enough to detect all dietary elements. The difference in breadth could relate to differences in diet on a given day, differential preservation of consumed materials as they traveled through the gut, or could relate to the difference in age between the samples. Taxa identified as unique to a component in this thesis are not necessarily absent from the others. Additionally, while relative abundances of materials were presented, they may not reflect the actual importance of each taxon in the diet.

Broadly speaking, most of the floral traces are too general to provide specific dietary information. Rather, trends can be suggested by the kinds of plants contained in the identified families. For components V and III, the presence of *Atriplex* and Asteraceae suggests a reliance on small seed resources, as many taxa from Chenopodiaceae and Asteraceae provide edible seeds. However, additional taxa from Asteraceae are used for their stems, leaves, and roots for dietary, medicinal, and other

purposes. For example, the roots of greater rabbitbrush can be chewed to produce gum, the wood of sagebrush burned for fire, and the leaves and seeds of arrow-root eaten (Table 9; Chamberlin 1911; Steward 1938). These kinds of resources may be collected at different times of the year, may have been consumed immediately or stored, or may have been unintentionally ingested. Genus and species level designations are needed to answer questions regarding seasonal resource use, habitat exploitation, and dietary components, but these resources do suggest the inhabitants of BER foraged in both upland and lowland contexts throughout the Holocene. The presence of *Pinus* could relate to the consumption of pine nut and utilization of resources found in the pinyon-woodlands. Some taxa appear to correlate to environmental trends. The presence of Poaceae in Component III compared to its absence in Component V may be a factor of the expansion of grasses in the late Holocene as compared to the middle Holocene (Grayson 2011; Hockett 2005, 2015, Kelly 1997). Overall, the small sample size and family-level designations of floral taxa make the genetic results more speculative when relating them to subsistence without any other forms of evidence. The faunal data, however, yielded species level designations. This is to be expected, as faunal genomes are often more widely studied than floral ones, suggesting that metabarcoding may have higher resolution for faunal species.

The Macroremains Data: Ethnographic Accounts

All identified items among the macroremains have known ethnographic uses. The seeds of both *Amaranthus* and goosefoot are present in the ethnographic record as

known dietary elements (Table A1; Chamberlin 1911; Steward 1938; Rhode 2002). Possible saltbush species include *Atriplex confertifolia*, *A. truncata*, and *A. canescens*; regardless of the exact species the seeds were a known food source (Chamberlin 1911; Steward 1938; Rhode 2002), as were Indian ricegrass seeds (Table A1; Rhode 2002). Prickly pear would have been eaten after removing the spines and roasting the joints over coals (Chamberlin 1911; Steward 1938; Rhode 2002). Sagebrush, however, is less often ethnographically consumed. One widely utilized species was *A. tridentata*, which was used to make fire or food storage, while the leaves had medicinal properties (Steward 1938; Rhode 2002). The seeds were generally eaten when food was scarce (Table A1). Other species include *Artemisia biennis*, *A. discolor*, *A. trifida*, and *A. dracunculoides*. While the faunal elements are unidentified, they indicate bird consumption across all three components, due to the presence of feathers, shell, and hollow bone fragments. The stones, often present in the smaller fractions, could be a result of seed processing with ground stones or from environmental contamination, and the charcoal indicates consumption of cooked foods.

Conclusions: The Macroremains Data

All but one sample provides evidence for a human source, as sample 5 was the only coprolite identified as having a canine depositor based on both color and contents. Those samples that turned the rehydration liquid dark and opaque did contain contents indicative of an omnivorous diet or of cooking and processing, supporting liquid color as a potential identification method. However, the samples that caused the liquid to turn a

lighter color could not be discounted, meaning if traditional identification methods are used, multiple data points should be considered. When looking at possible diet breadth, Component III contained the largest number of identified taxa and unidentified contents. Component V contained the next-largest diet breadth, while Component II displayed the least.

When looking at the contents as a whole, the inclusion of pickleweed and other small seeds along with bird remains in all three components suggests continued reliance on both dryland small seeds and more wetland resources throughout the Middle and Late Holocene. While the amount of pickleweed in each component does not necessarily relate to its dietary importance, it was more abundant in Component V than in components III and II. Other resources in these components follow the same trend, displaying the utilization of resources from both upland sagebrush and lowland shadscale communities, along with use of pinyon-juniper woodland communities. As mentioned above, the presence of charcoal and stone could relate to cooking and processing methods.

Generally, morphologically distinct floral remains in these samples were identifiable, with some left unidentified or at the genus level either due to the lack of a comparative sample or inexperience of the researcher. These items should be identifiable once references are acquired. Identification of the faunal remains may be possible, but the bone is highly fragmented and may not contain morphologically distinct parts. This means additional taxa may be present that have not yet been identified. A larger difficulty in processing these samples and determining their full contents and

abundances was the researcher's inability to disaggregate all materials in some of the samples. This was especially true for samples containing large amounts of small seeds, which were very fragmented and surrounded by coprolite matrix. Full separation of these samples was not possible without damaging them. Additionally, it is possible rarer traces were missed when scanning the 250-micron fraction, so the identified macroremains presented here may be a partial list of the full diversity present in the samples.

Genetic Versus Macroremains Data

In regard to detecting the largest number of unique taxa, DNA outperformed macroremains analysis by identifying 17 compared to eight. However, the majority of these identifications were to the family level and the specific taxa are unknown: this is likely due to the lack of comparable reference sequences. Many of the flora present in the ethnographic record and detected in the macroremains do not have full reference sequences in GenBank. Of the flora with partial references, many do not include the chloroplast trnL intron used in this thesis. DNA identifications rely on these references, and without them identification to lower taxonomic levels may not be possible. Only fauna and tobacco, a model organism, were identified to the species level. When considering genus and species designations, both macroremains and DNA did approximately the same in regard to floral taxa (Table 9, Figure 6).

Table 9 Ubiquity of Floral Taxa Identified to the Genus or Species Level in DNA and Macrofossil Data

		V		III		II	
Genus	**Species**	**DNA**	**Macros**	**DNA**	**Macros**	**DNA**	**Macros**
Abies		0/5	0/5	2/3	0/3	0/2	0/2
Allenrolfea	*Occidentalis*	0/5	4/5	0/3	1/3	0/2	2/2
Amaranthus		0/5	1/5	0/3	0/3	0/2	0/2
Artemisia		0/5	0/5	0/3	1/3	0/2	0/2
Atriplex		1/5	0/5	3/3	1/3	1/2	0/2
Chenopodium	cf. *nevadense*	0/5	1/5	0/3	0/3	0/2	0/2
Corispermum		0/5	0/5	1/3	0/3	0/2	0/2
Ephedra		1/5	0/5	1/3	0/3	0/2	0/2
Lappula		0/5	0/5	0/3	0/3	1/2	0/2
Nicotiana	*attenuata*	0/5	0/5	1/3	0/3	0/2	0/2
Cf Opuntia		0/5	0/5	0/3	1/3	0/2	0/2
Oryzopsis	cf. *hymenoides*	0/5	0/5	0/3	1/3	0/2	0/2
Pinus		2/5	0/5	2/3	0/3	0/2	0/2
Total Taxa		3	3	6	5	2	1
Unique taxa		3	3	5	4	2	1

Pickleweed, pigweed, ricegrass, sagebrush, prickly pear, and goosefoot were found among the macroremains, while *Ephedra* sp., *Pinus* sp., *Abies* sp., *Corispermum* sp., *Lappula* sp., and tobacco were found in the genetic remains. Saltbush was the only shared taxon. The next step is to determine if the unique floral macroremains could be represented by any of the taxonomic families found in the genetic data. Surprisingly, with the exception of a few samples, the genetic and macroremains data do not appear to overlap (Figure 6). Three remains in sample 7 could be linked to the genetic content. These are Indian ricegrass, which could be represented by reads attributed to Stipeae; *Atriplex*, the only shared genus between both data types; and sagebrush, which could be represented by the reads attributed to Asteraceae, Asteroideae, and Artemisiinae. A second possible connection is in sample 8, which contains DNA attributed to jackrabbit and traces of hair. Beyond those examples, one final connection may be the presence of large amounts of unassigned reads across all three components that resemble cheno-ams.

Some of these may be pickleweed, especially in samples such as 13 where that is the predominant macrofossil identified.

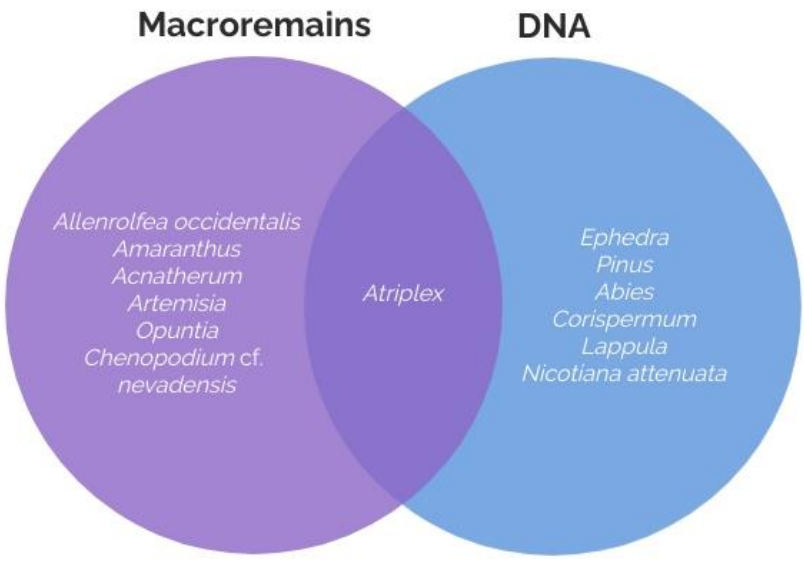

Figure 6 Floral taxa identified to the genus level using macroremains and DNA.

This lack of overlap could relate to both the types of plants consumed and the DNA subsampling methods. When subsampling for DNA analysis, care was taken to avoid visible macroremains to allow for their analysis later on and to avoid destroying potentially informative specimens. This means genetic data are primarily composed of traces found in the overall coprolite matrix. Some of the families found in the genetic data could relate to the more "invisible" dietary contents that are often lost during digestion or made unidentifiable. These potentially include leaves, shoots, roots, fruits, and meats. One example is the discovery of Apiaceae in components V and II samples. The utilized parts often include leaves, underground structures, and young shoots that are roasted, boiled, and sometimes cached (Table A1; Chamberlin 1911; Steward 1938).

While the fibrous elements may preserve, any definable morphological traits may not. Some genetic traces may also link to non-dietary items present in highly fragmented or trace amounts in the macroremains. A key example is from sample 7 in Component III. Reads attributed to Dryadoideae could come from only one of four genera, one of which is mountain mahogany. The wood was a known fuel source, and the sample contained the only non-trace amounts of charcoal in the whole coprolite assemblage. If those reads are attributed to mountain mahogany, it demonstrates engagement with a high-elevation environment and non-dietary plant use.

The macroremains, on the other hand, are composed primarily of hard, less digestible items such as seeds, fiber, bone, hair, feather, eggshell, and cactus spines and glochids. The advantage of these items is that when they possess morphologically distinct traits they can be identified. However, just as with the DNA data, they only represent a portion of the whole diet. Without specific taxonomic identifications, much of the genetic data are open to interpretation, but there is potential for studying how different materials preserve in the genetic and macroscopic records.

The methods also differ with defecator identification. Using molecular and traditional identification methods gave different but overlapping results for which samples were human and non-human. Molecular methods identified five human coprolites, while traditional methods identified between six and nine when accounting for color and contents. If only rehydration liquid color is considered, then three samples are identified as human based on both methods. These are samples 7, 10, and 11. However, when taking coprolite contents into account, samples 8 and 9 are also labelled

as possibly human by both. Additionally, sample 5 was identified as a probable dog coprolite based on genetic and macrofossil data. The rest of the samples, 4, 6, 12, and 13, looked human based on traditional methods, but no faunal DNA was detected in any of them. Based on previous studies such as those described in Chapter 1, defecator DNA is expected in coprolites and often used for identification. The lack of defecator DNA does not necessarily mean it is not present in the samples. It is possible that a different amplicon, targeted sequencing for particular species, or shotgun sequencing would result in defecator detection. What these results suggest is that the presence of human DNA can be a good indicator of a human source, just as the presence of dog DNA and no human DNA can indicate a canine source. However, because the traditional methods identified a larger number of potential human samples, those samples that do not contain human or other faunal DNA in a metabarcoded data set should not be automatically labelled as non-human.

When considering what the coprolite contents say about diet, the results in this thesis correlate to previously published data about BER and other sites in the region. Coprolites from this study and from previous work on BER show a high reliance on small seeds during the Middle Holocene and a gradual decrease in the Late Holocene as temperatures cooled. These dryland resources would have been supplemented by wetland resources when available. This is seen in the macroremains data by the ubiquity of pickleweed, and possibly in the genetic data by the presence of reads associated with cheno-ams and other families with taxa that produce small seeds such as Asteraceae. The most ubiquitous dietary plant in the Middle Holocene at BER is pickleweed. Its

collection and processing would have been economically taxing, as the inhabitants of BER would have needed to travel up to 6 km to reach the nearest playa margins (Albush 2010; Rhode 2008). Additional macroremains found in coprolites include small seeds of dropseed and beeweed, cactus spines, unidentified plant fiber and epidermis, and faunal elements identified as cottontail, bird, fish, pronghorn, insect, and small mammal (Albush 2010). Stratum 14 hearth features additionally contained goosefoot, shadscale, ricegrass, cactus seeds and pads, pinyon pine cones and nut hulls, and cattail seeds (Rhode 2006), while additional faunal elements were jackrabbit, sagegrouse, and other small animals (Hockett 2007). When looking at the contents of samples from this thesis, overlapping items from the macroremains include pickleweed, goosefoot, plant fiber and fragments, bones, and bird remains. A unique set of macroremains from this study is pigweed, which is known ethnographically to have been consumed by Gosiute people (Chamberlin 1911; Rhode 2002), but it can be considered in the same category as other small seeds, meaning no unexpected or surprising macroremains were found. Genetic faunal traces are jackrabbit, while floral traces could relate to additional small seeds, pine, and juniper.

The record at BER of Late Holocene subsistence still shows reliance on small dryland seeds. Remains found in coprolites include small seeds from pickleweed, bluegrass, and Indian ricegrass, pinyon pine, Solanaceae, cactus spines, shadscale, unidentified plant fibers and epidermis, and various faunal remains including rodent, ungulate, fish, insect, reptile, and pronghorn hair (Albush 2010). Botanicals further include prickly pear, bulrush seeds, shadscale, and a maize fragment (Rhode 2006).

Overlapping taxa from this study include pronghorn, Poaceae, tobacco (while the Solanaceae seed is an unknown taxon, it shares a family with tobacco), shadscale, pine, Indian ricegrass, prickly pear, plant fibers and epidermises, various small seeds, avian faunal traces, and insect remains.

Overall, the samples in this thesis display a reliance on small seed resources that appears greater in the Component V sample than in components II and III. Usage of both desert and some wetland resources is present across the samples. While Indian ricegrass is found in samples from both the Middle and Late Holocene, the presence of grasses in only the Component II and III samples could related to the expansion of grasses, and genetic reads likely map to additional species. The contents further correlate to published data through the presence of pickleweed and the utilization of both large and small mammal resources along with bird, the use of various hard-coated seeds, and upland pine nuts and fruits.

CHAPTER V

CONCLUSIONS

This thesis allows for the direct comparison of established methods used for dietary reconstruction in paleofecal research. It builds upon existing data while adding new genetic traces to what is known about diet at Bonneville Estates Rockshelter, and it will aid future researchers in choosing the kinds of analyses to perform when looking for certain flora and fauna. Furthermore, it addresses whether or not the presence of human DNA or known dietary elements can be used as a reliable way to identify the depositing organism. The results from this study correlate to previously published data on BER, showing a reliance on small dryland seed resources in the Middle Holocene that declines in the Late Holocene. However, it is important to note that the sample size used in this study is exceptionally small (Table 3; Table 6), so they likely contain less material overall than samples in previous studies.

Wetland resources were utilized when available but not in excess of the dryland resources regardless of environmental context. This suggests a subsistence strategy focused on reliable resources even if they were not the most economically rich. When comparing taxa between components, it was seen that there was a fair amount of overlap between components V and III, with less overlap with Component II. This is likely a feature of there being fewer taxa identified in Component II samples in general, possibly because fewer coprolites were analyzed. The results from this study also support the multiproxy study of coprolites. DNA metabarcoding and macroremains analysis are

complementary studies, as the data provided with each was largely unique with very little taxonomic overlap. This suggests the methods should be used in conjunction if possible, and additional analyses added when available. When it comes to defecator identity, confirmation by multiple methods may be better than using one.

Future Work

Future work would focus on increasing the sample size, confirming the defecator through additional means, and increasing the taxonomic resolution of both the genetic and macroremains data. One additional analysis needed is radiocarbon dating to confirm sample placement in the chronology and to see how, within components, the various samples relate temporally with each other. The addition of shotgun sequencing may be used to confirm the defecator, detect additional dietary taxa, and detect changes in the gut microbiome between components. Comparisons can also be made between the shotgunned, metabarcoded, and macroremains data sets to provide further insight into taxonomic preservation and potentially detect additional "invisible" taxa.

Increasing resolution would start with collecting modern seeds representing species found in the eastern Great Basin with known ethnographic uses, followed by those expected in the environment. Some would be saved in a comparative collection for macroremains identification while DNA would be extracted from the excess. The modern references would be sequenced and compiled into a custom database, and

ancient reads compared to it, potentially allowing for additional genus and species level identifications of floral taxa. For the faunal data, remains could be sent to a zooarchaeologist for identification. Samples that are too fragmented or lack distinguishing characteristics can be ground for DNA metabarcoding. The 250-micron portion of each coprolite could be fully sorted to see if any rare taxa were missed, as well as to get a better idea of inter-sample composition.

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APPENDIX A

Table A1 Summary of Ethnographic Uses for Possible and Recovered Plants

Family	Scientific Name	Common Name	Food	Medicine	Other	Source
Apiaceae	*Cymopterus longpipes*	Spring parsley	Boil leaves			Chamberlin 1911; Steward 1938
	Cymopterus monatus		Underground structures			Chamberlin 1911
	Lomattium multifida		Young shoots	Apply paste to wounds		
	Carum gairdneri	Yampah	Roast, boil roots; cache for winter			Chamberlin 1911; Steward 1938
	Peucedanum graveolens			Root pulp for sore throat		Chamberlin 1911
	Angelica pinnata			Roots		Chamberlin 1911
	Ferula multifida		Young shoots	Root paste to wounds		Chamberlin 1911
Asteraceae	*Balsamorrhiza sagittata*	Arrow-root	Boil leaves, beat seeds	Root paste to wound		Chamberlin 1911
	Artemisia tridenta	sagebrush	Seeds when food scarce	Leaves for fevers, rheumatism, coughs, colds	Fire, cover stored foods	Rhode 2002; Steward 1938
	Artemisia biennis, discolor, trifida, dracunculoides		Seeds			Chamberlin 1911; Steward 1938
	Balsamorrhiza hookeri		Seeds beaten from head			Chamberlin 1911
	Wyethia amplexicaulis, Gymnolomia multiflora, Helianthus		Seeds beaten from heads	Steep roots, reduce swelling		Chamberlin 1911
	Cnicus eutone, drummondi, undulatus	thistle	Stems	Treat wounds, cuts, sores		Chamberlin 1911
	Senecio				Latex dried for gum	Chamberlin 1911
	Bigelovia douglasii (now Chrysothamnus viscidiflorus)	Greater rabbit-brush			Chew roots to produce gum	Chamberlin 1911; Rhode 2002; Steward 1938
	Achillea millefolium	Common yarrow		Steep, treat rheumatism, biliousness, headache		Chamberlin 1911; Steward 1938
	Ambrosia psilostachya	ragweed		Sore eyes-steep leaves and bandage eyes		Chamberlin 1911

	Antennaria dioica			Steep, treat snow-blindness	Chamberlin 1911
	Brickelia grandiflora			Roots	Chamberlin 1911
	Chaenactis douglasii			Paste, treat aches and soreness	Chamberlin 1911; Steward 1938
	Crepis glauca		Leaves		Chamberlin 1911; Steward 1938
	Erigeron grandifloras, macranthus, ovalifolium, villiflorum			Medicinal purposes, etc.	Chamberlin 1911
	Grindelia squarrosa			Roots, treat cough	Chamberlin 1911; Steward 1938
	Gymnolomia multiflora		Seeds		Chamberlin 1911
	Helianthus		Seeds, also source of oil Seeds beaten out of heads or usual basket collecting		Chamberlin 1911; Steward 1938
	Lactuca ludoviciana		Ate leaves		Chamberlin 1911
	Anisocoma acaulis		Cook greens with hot rocks		Steward 1938
	Aster canescens		Seeds of related maybe	Primary use	Steward 1938
Boraginaceae	*Lithospermum*		Seeds	Root tea, kidney trouble strong diuretic	Chamberlin 1911
	Amsinckia tessellata		Seeds		Chamberlin 1911; Rhode 2002
Brassicaceae	*Sisymbrium canescens*	Hedge muster	Eat seeds, gathered in usual way, grind and mix with snow in winter to eat as confection		Chamberlin 1911
	Nasturtium	watercress	Eat leaves		Chamberlin 1911; Steward 1938
	Stanleya pinnata	Desert prince's plume	Greens in spring		Rhode 2002
	Thelypodium integrifolium	Entireleaved thelypody	greens		Rhode 2002
	Descirainia pinnata	Tansy mustard	Greens in spring, seeds gathered in summer		Rhode 2002
	Streptanthus cordatus	Heartleaf twistflower	Seeds in summer, greens		Rhode 2002

Chenopodiaceae	*Atriplex confertifolia, truncata, canescens*	Saltbush, shadscale	Seeds	Leaves for cuts	Fire, arrow shafts	Chamberlin 1911; Rhode 2002; Steward 1938
	Chenopodium capitatum, leptophyllum, rubrum	Goosefoot, pigweed	Seeds, leaves			Chamberlin 1911; Rhode 2002; Steward 1938
	Salicornia herbaceae		Make meal from seeds			Chamberlin 1911; Steward 1938
	Amaranthus		Seeds			Chamberlin 1911; Steward 1938
	Eurotia lanata	White sage		Fevers, used like Artemisia		Chamberlin 1911; Steward 1938
Cactaceae	*Opuntia*	Prickly pear	Spines removed, joints roasted in hot coals			Chamberlin 1911; Rhode 2002; Steward 1938
Cupressaceae	*Juniperus californica*	cedar	Sometimes boil and eat berries	Leaf tea for colds, coughs, pulmonary bronchial affections, sometimes mixed with sagebrush	Fire, make winter lodges, bark used for storage pits	Chamberlin 1911
	Juniperus osteosperma	Utah juniper	berries	Treat colds, coughs, asthma, swelling, aches, splints, use twigs and berries	Burned for purification, make bows, fuel, construction, seeds as beads	Chamberlin 1911; Rhode 2002
Pinaceae	*Abies menziessi*	balsam			Gum, pitch, etc.	Chamberlin 1911
	Pinus monophylla	Singleleaf pinyon pine	Nut important-collect yearly; parthially char cones, beat out roasted nuts. Eat directly or grind	Boil gum, treat worms and intestinal parasites	Pitch for waterproofing, adhesive, gum	Chamberlin 1911; Rhode 2002; Steward 1938
Poaceae	*Cinna arundinacea*	Reed grass	Seeds			Chamberlin 1911; Steward 1938
	Elymus canadensis or sibiricus		Seeds			Chamberlin 1911; Steward 1938
	Festuca tenella, ovina		Seeds			Chamberlin 1911; Steward 1938
	Poa californica,	Blue grass	Seeds			Chamberlin 1911;

	tenuifolia, pratensis, nevadensis					Steward 1938
	Oryzopsis cuspidata	Mountain rice	Seeds or grain			Chamberlin 1911
	Bromus breviaristatus	Brome grass	Seeds			Chamberlin 1911
	Agropyron	Wheat grass	seeds			Chamberlin 1911; Steward 1938
	Stipa	needlegrass	seeds			Chamberlin 1911; Rhode 2002; Steward 1938
	Eriocoma hymenoides	Indian ricegrass	seeds			Rhode 2002
Rosaceae	*Cercocarpus ledifolius*	Mountain mahogany		Treat burns, charr green wood, powder charcoal, mix with water, apply to burn	Fire, bows	Chamberlin 1911; Rhode 2002; Steward 1938
Solanaceae	*Nicotiana attenuata*	tobacco			Smoke	Chamberlin 1911; Rhode 2002; Steward 1938
	Nicotiana quadrivalvis	tobacco				Chamberlin 1911

*List is not exhaustive

Table A2 Read Counts for Each Sample and Taxa

Family	**Lowest Taxonomic Level**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Hominidae	*Homo sapiens*	0	0	0	11244	4197	24232	10663	36020	0	0
Antilocapridae	*Antilocapra americana*	0	0	0	0	7210	0	0	0	0	0
Canidae	*Canis familiaris*	0	31841	0	0	237	0	0	0	0	0
Leporidae	*Lepus californicus*	0	0	0	0	0	7412	0	0	0	0
Apiaceae	Apioid Superclade	4	0	0	0	0	0	0	26873	0	0
	Acronema Clade	0	0	0	0	0	0	0	48	0	0
Asteraceae		0	0	0	337	0	0	11218	0	0	0
	Asteroideae	0	0	0	294	0	0	0	0	0	0
	Artemisiinae	0	0	0	673	529	0	0	0	0	0
	Anthemideae	0	0	0	0	90	0	0	0	0	0
Boraginaceae	*Lappula*	19391	0	0	0	0	0	0	0	0	0
Brassicaceae		0	0	0	0	0	26	0	0	0	0
Chenopodiaceae	*Atriplex*	6	0	11689	4887	4356	152	0	0	0	0
	Corispermum	0	0	0	166	0	0	0	0	0	0
Cupressaceae		0	0	0	0	2830	38	0	0	0	0
Ephedraceae	*Ephedra*	0	0	0	0	2001	19	0	0	0	0
Euphoribaceae	***Hevea***	**0**	**227**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Pinaceae	*Abies*	0	0	0	635	3241	0	0	0	0	0
	Pinus	0	0	0	0	787	0	0	6149	0	0
	Pinus subgen	0	0	0	0	10204	47	0	0	0	0
Poaceae	Poeae Chloroplast Group 2	0	0	0	1671	0	0	0	0	0	0
	Stipeae	0	0	0	9402	0	0	0	0	0	0
	Triticodae	0	0	0	0	490	0	0	0	0	0
	PACMAD Clade	0	0	0	0	2490	0	0	0	0	0
	Cenchrinae	0	0	0	0	7	0	0	0	0	0
Proteaceae		**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**5149**
Rosaceae	Dryadoideae	0	0	0	3473	0	0	0	0	0	0
Solanaceae	*Nicotiana attenuate*	0	0	0	27	0	0	0	0	0	0
Other	No hits	19297	5119	3464	4873	7829	186	0	2649	1097	189
	Not assigned	0	15561	8379	1639	405	11071	0	0	12274	13630