1-5-2008

Evaluating chloroplast DNA in prehistoric Texas coprolites: medicinal, dietary, or ambient ancient DNA? (Galley Proofs)

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Evaluating chloroplast DNA in prehistoric Texas coprolites: medicinal, dietary, or ambient ancient DNA?

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Abstract

Molecular analysis of coprolites from Hinds Cave, Texas recovered chloroplast DNA sequences. The sequences were interpreted as evidence of diet. We analyzed 19 Hinds Cave coprolites to evaluate the potential sources of the chloroplast DNA (cpDNA) and compared our results to previous studies. This review shows that some cpDNA sequences could be from well-known prehistoric plants foods. Some other sequences could have come from ambient plant material in the guts of small animals eaten by humans in antiquity. Using pollen concentration analysis, we identify sources of ambient plant material which could have been inhaled or imbibed. It is even possible that cpDNA sequences are from proplastids within ambient pollen grains themselves. However, three sequence types cannot be explained as resulting from only dietary or ambient sources. We suggest instead that these might be from medicinal or hallucinogenic plants. We compared these three sequences to existing sequences in the GenBank. We found that these sequences are 100% matches for *Rhamnus*, *Fouquieria*, and *Solanum*.
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Keywords: Molecular biology; Pollen concentration; Zooarchaeology; Ambient plant residue; Coprolite

1. Introduction

In the *Proceedings of the National Academy of Sciences*, Poinar et al. (2001) reported on the recovery of ancient DNA from three Late Archaic Period human coprolites (desiccated feces) from Hinds Cave, Texas. They found a diversity of ancient DNA sequences, including eight chloroplast DNA (cpDNA) sequences. Five cpDNA sequences were from plant taxa that were not identified in macrofossil analysis of the same coprolites. They interpreted these five sequences as evidence of dietary plant use. The discovery highlighted the value of molecular biology in ancient diet reconstruction. We will discuss these five sequences, and their origins, in this paper.

The five cpDNA sequences in question are Asterales/Asteraceae, Ericales/Fouquieriaceae, Fagales/Fagaceae, Rhamnales/Rhamnaceae, and Solanales/Solanaceae. Poinar et al. (2001) suggested that *Helianthus* or other genera might be represented by Asteraceae cpDNA, *Fouquieria* by Fouquieriaceae cpDNA, *Quercus* by Fagaceae cpDNA, *Karwinskia*, *Condalia*, or *Colubrina* by Rhamnaceae cpDNA, and *Nicotiana*, *Physalis*, *Lycium*, or *Datura* by Solanaceae cpDNA. At the time of their research, 2000, sequences for these genera were unavailable for comparison in GenBank.

The cpDNA sequences are of particular interest because they include some plant taxa that have never been encountered in dietary residue in any past study of Hinds Cave coprolites. Over 200 human coprolites from Hinds Cave have been the focus of past studies dating from the Early Archaic to the Late Archaic (Danielson and Reinhard, 1998; Dean, 2006; Edwards, 1990; Reinhard, 1988, 1992; Stock, 1983; Williams-Dean, 1978). These studies show a continuity of diet from Early to Late Archaic times with relatively little dietary variation between periods (Reinhard, 1992). Of 25 taxa encountered in

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the coprolites reviewed by Reinhard (1992), significant variation between different studies was noted for only four taxa; wild onion, walnut, mesquite and dropseed grass. These analyses have revealed a general long-term pattern of plant use at Hinds Cave through analysis of pollen, phytoliths, and macroscopic plant residues such as fibers and seeds.

Microfossil and macrofossil coprolite analyses are frequently concerned with issues of prehistoric or modern ambient plant residue contamination of samples (Chaves and Reinhard, 2006; Reinhard et al., 2006a; Sobolik, 1988). For this paper, ambient plant residue refers to plant material inadvertently incorporated into coprolites in ancient times by several means. These range from consumption of ambient plant residue from whole animals or insects, plant residue inadvertently swallowed while processing plant materials for non-dietary purposes, and consumption of pollen-carrying plant residues via inhalation and swallowing. After deposition, ambient plant residue from cave deposits could infiltrate the coprolite matrix.

At Hinds Cave, cpDNA from ambient plant residue can come from several likely sources evaluated in this paper. Ancient inhabitants of the cave ate many species of small animals whole as evidenced by analysis of animal bones in the human coprolites (Reinhard et al., 2006c; Dean, 1978). This introduces vegetation from the prey digestive tracts into human digestive tracts (Reinhard et al., 2002, 2006c). Secondly, consumption of aqueous plant solutions, i.e. teas, can introduce plant residue into intestinal tracts (Chaves and Reinhard, 2006; Reinhard et al., 1991). Consumption of ambient, pollen-associated plant material with food, drink, or inhalation might add cpDNA sequences into coprolites. Finally, a more remote possibility is that inhalation of pollen could have introduced cpDNA sequences into the coprolites. Although chloroplasts are not present in angiosperm pollen, proplastids sometimes are. Proplastids are DNA-carrying, organelle precursors to chloroplasts. They can be in the generative and vegetative cells in pollen grains (Bennett and Parducci, 2006; Pacini et al., 1992; Sangwan and Sangwan-Norreel, 1987; Schmitz and Kowallik, 1987; Sodmergen et al., 1994). Recently, cpDNA was recovered from ancient *Fagus* pollen (Paffetti et al., 2007).

To evaluate the potential sources of ambient plant residue, we undertook an analysis of 19 Middle Archaic Hinds Cave coprolites. We compared the results of this analysis with Edward's (1990) analysis of Late Archaic Hinds Cave coprolites and Dean (2006);(Williams-Dean, 1978) analysis of Early Archaic coprolites. The coprolites analyzed by Edwards date between 2100 and 600 B.C. The coprolites analyzed by Dean date between 7710 ± 80 and 7590 ± 80 B.C. The coprolites we analyzed are from Hinds Cave (41VV456) B-1, Lens 5 (2560–2810 B.C.) and B-1 Lens 7 (3680 ± 80 B.C.). These are uncorrected dates. Specific provenience information of the collection bags state B-1 B6-XI (K), B-1 Lens 5, and B-1 Lens 7. The stratigraphy of Hinds Cave is summarized in several unpublished sources (Lord, 1984; Saunders, 1986; Shafer and Bryant, 1977). However, this subject has been well treated on the Texas Beyond History Hinds Cave website. For consideration of stratigraphy and provenience, (see http://www.texasbeyondhistory.net/hinds/explore.html).

It would have been ideal to extract pollen from the exact coprolite samples analyzed for cpDNA. In the most recent methods of coprolite analysis, macroscopic remains, phytoliths, parasites, and pollen are recovered sequentially from the same coprolite sample (Reinhard, 1988; Reinhard et al., 2006a, b) or from two separate subsamples removed from the same coprolite (Reinhard et al., 2002). The goal of this analysis was to assess whether cpDNA sequences from the small sample analyzed by Poinar et al. (2001) could have been derived from intentional dietary use or were signals of ambient plant material. We could not access the same coprolites analyzed by these authors. So we based our evaluation on the independent analyses of larger series from the site. In this way, our aim was to detail whether or not these author's conclusions were valid based on a comprehensive review of 169 coprolites analyzed by Dean (1978), Edwards (1990), and ourselves in this study.

The continuity in general diet between Early to Late Archaic periods (Reinhard, 1992) has been previously demonstrated. Thus, including all periods for comparison with the molecular analysis of Late Archaic coprolites is justified. Furthermore, we have the potential of demonstrating that ambient cpDNA was a long-existing aspect of lower Pecos environment which poses a problem for molecular interpretation of other coprolites from other Archaic periods.

2. Materials and methods

Five-gram fragments of Hinds Cave coprolites were removed from the interior of 19 coprolites that were brushed clean of extraneous cave sediments. The coprolites were rehydrated in 0.5% trisodium phosphate for 48 h. Five *Lycopodium* tablets (batch 201890, each containing $11,300 \pm 400$ *Lycopodium* spores), were added to the rehydrating coprolites. The rehydrated coprolites were then screened through a 300 micrometer mesh with a jet of distilled water. The water and microscopic residues that passed through the screen were collected in a beaker and concentrated by centrifugation. The concentrated microscopic residues were then washed in glacial acetic acid and processed for 10 min in acetolysis solution (1 part sulfuric acid to 8 parts acetic anhydride) at 98 °C. The residues were then washed in glacial acetic acid, and subsequently with distilled water. The residues were then treated with approximately 45% hydrofluoric acid for 24 h at room temperature. The residues were then washed three times with distilled water, concentrated by centrifugation, and then transferred to 2 dram vials with glycerin. Microscopic examination was done at 400× and 1000× magnification. A minimum of 200 pollen grains was counted for each sample. Pollen was identified by comparison to reference samples of lower Pecos pollen curated in the Palynology Laboratory, Department of Anthropology, Texas A&M University, and with samples collected by Reinhard in 1991 and curated in the School of Natural Resources, University of Nebraska-Lincoln. The processed samples are stored in glycerin. *Lycopodium* spores were counted as they were encountered during the pollen count. The pollen concentration values were calculated by

the following formula in terms of number of pollen grains per gram of coprolite (modified from Maher, 1981).

Pollen concentration

$$= ([\text{pollen grains counted}/ \text{marker grains counted}] \times \text{number of markerpollen grains added})/ \text{coprolite weight}.$$

The pollen concentration data were then used to determine the approximate numbers of pollen grains from identifiable plant taxa in the coprolites. The pollen data were compared with the list of taxa represented by cpDNA sequences to assess which sequences represent diet, which sequences represent ambient plant residue, and which sequences could be derived from both diet and/or ambient plant residue.

An analysis of animal remains in the coprolites was done to evaluate the potential of introduction of ambient plant residue through consumption of entire small lizards, rodents, fish, and insects such as grasshoppers. The plant diets of the animals were researched to assess whether they eat the plant taxa found by Poinar et al. (2001).

We compared the *rbcL* gene sequence of three taxa recovered by Poinar et al. (2001) with GenBank sequences (October, 2007). Specifically, the consensus sequence of DNA sequences of clones from Rhamnaceae, and Solanaceae, and the unique clone sequence of Fouquieriaceae identified by the authors were chosen. The best hits defined by maximal identity of a BLAST search were considered most likely plant origins.

3. Results

Animal remains were commonly found in the coprolites. One sample contained a rodent tooth. Bone fragments of rodents were found in 16 samples. The absence of burning or other evidence of heat alteration of the bone indicates that these animals were eaten raw or in a semi-cooked state. Lizard scales were present in two samples. Fish bones and scales were present in one sample and fish scales were present in another sample. Non-human hair was present in six samples. Masticated insect fragments unidentifiable to family were found in 13 coprolites and masticated grasshopper fragments were present in two coprolites. Recent analyses report an association of trace amounts of choridoid, festucoid, and panicoid grass phytoliths (Reinhard et al., 2002) and fungal spores of animal dietary origin (Reinhard et al., 2006c) in coprolites with animal bone. These are best interpreted as food residue from the intestinal tracts of prey animals eaten whole. Previous phytolith analysis of these Hinds Cave coprolites revealed traces of choridoid, festucoid, and panicoid phytoliths (Danielson and Reinhard, 1998). Thus, the coprolites from Hinds Cave present substantial evidence that whole animals were consumed. This evidence includes hair, teeth, bone, and phytoliths that are best explained as the result of eating small animals in their entirety.

Pollen analysis revealed 47 identifiable taxa of flowering plants in the 19 coprolites. In addition, one type of fern spore was identified. Five pollen types of unknown origin were also found. Therefore, a total of 53 palynomorph types were represented. Of the 47 identifiable flowering plant taxa, 22 occurred only in trace amounts (500–25,000 grains of pollen per gram of coprolite). Thus, 25 taxa were found in high numbers in one or more coprolite (in excess of 100,000 grains of pollen per gram).

The 25 taxa of greater numerical occurrence are listed in Table 1. In a few coprolites, the numbers of pollen grains per gram of certain taxa are very high, exceeding 100,000 grains per gram, and indicate intentional consumption of plants. *Agave*, Cactaceae, *Dasylirion*, and higher Poaceae counts are among these.

The remaining 21 taxa occur in numbers that are consistent with prehistoric ambient pollen inclusion in feces as discussed in recent papers (Reinhard et al., 2002, 2006a, b). Low Poaceae, Cheno-Am, and Low Spine Asteraceae made up the majority of the Lower Pecos pollen rain in prehistory (Dean, 2006; Edwards, 1990; Reinhard, 1988; Sobolik, 1988; Stock, 1983; Williams-Dean, 1978). Therefore, these pollen types have an ambient source. *Quercus*, *Larrea*, *Celtis*, *Eriogonum*, and *Ephedra* are moderately common ambient types. However, when *Larrea* and *Ephedra* occur in high concentrations, their use as a medicinal tea can be interpreted (Reinhard et al., 1991).

Table 2 presents a comparison of common ambient pollen taxa found in this study compared to Dean (2006) and Edwards (1990). These data show that ambient plant material from *Alnus* (alder), Asteraceae (sunflower family), *Celtis* (hackberry), Cheno-am (goosefoot family and pigweed genus), *Ephedra* (mormon tea), *Juglans* (walnut), *Pinus* (pine), Poaceae (grass family), *Quercus* (oak), and *Ulmus* (elm) occurred in all Archaic time periods.

The comparison of the Fouquieriaceae, Rhamnaceae, and Solanaceae sequences recovered from Poinar et al. (2001) with GenBank presented mixed results (Table 3). The Fouquieriaceae sequence is a 100% match for a non-endemic species, *Fouquieria columnaris*. There is another *Fouquieria* species in the region *F. splendens* or ocatillo. It is more likely that the *Fouquieria* cpDNA comes from this species. The Rhamnaceae sequence is 100% consistent with two species of *Rhamnus*, or buckthorn. There are endemic species of *Rhamnus* in the region. It is also identical with *Sageretia* which is also present in the region. The Solanaceae sequence is 100% match for *Solanum lycopersicum* and *Lycopersicon esculentum* or the cultivated tomato. This is a mismatch for any species in the area. There is a wild solanaceous plant in the region, *Solanum triquetrum*.

4. Discussion

As shown in this analysis, the prehistoric inhabitants of Hinds Cave ate small animals in their entirety. Our findings support Dean's previous analysis of 100 Hinds Cave Coprolites (Williams-Dean, 1978). Reinhard et al. (2006c) presented a case that the consumption of small animals in their entirety was a common prehistoric hunter-gatherer subsistence component. They also present a case that identification of small

Table 1

Pollen concentration values for coprolites in terms of number of grains of pollen per gram of coprolite

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Acer	62	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Agave	312	17,947	259	0	413	0	17,363	564,435	0	0	130	0	0	0	342	0	242	782	90
Alnus	0	0	416	0	0	0	0	0	0	0	0	0	0	284	0	0	0	0	0
Aster. H.S	125	4985	83	87	310	2166	0	25,425	1640	0	173	2152	0	284	685	56	242	168	0
Aster. L.S	499	10,968	583	1527	9302	2166	827	1695	1640	547	691	0	377	851	2739	564	484	949	271
Brassicac.	125	0	0	44	0	0	0	0	0	0	475	0	0	0	0	0	0	56	0
Carya	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	90
Cactaceae	0	14,956	0	0	310	0	0	328,839	7108	0	259	0	0	0	0	0	0	112	90
Celtis	1122	7976	0	0	827	283	0	0	0	0	86	0	0	0	0	56	121	56	90
Cheno Am	249	997	500	654	3721	6027	0	0	547	547	86	0	0	142	342	790	363	112	0
Dasylirion	561	121,641	83	349	3411	0	776,393	0	324,784	0	734	0	3767	2837	1712	395	242	56	0
Ephedra	0	0	0	44	103	94	0	0	547	0	43	0	0	142	0	0	121	112	0
Eriogonum	0	1994	0	0	207	659	827	0	0	0	216	0	0	284	0	0	242	0	0
Euphorb.	0	997	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fabaceae	0	0	0	0	723	0	0	0	0	0	86	0	0	0	0	0	0	0	0
Fraxinus	0	0	0	0	0	0	0	0	0	547	0	0	0	0	0	0	0	0	0
Juglans	0	0	0	0	103	0	0	0	0	0	43	0	0	0	0	0	0	0	0
Larrea	62	0	0	305	0	377	0	0	0	0	0	0	0	142	0	0	0	168	0
Malvaceae	0	0	0	0	310	0	1654	33,900	0	0	0	1076	0	0	0	0	0	56	0
Pinus	62	1994	83	131	207	0	1654	0	0	0	43	0	0	567	0	0	0	335	0
Poaceae	1620	18,944	500	4145	8888	7722	0	11,865	0	659,410	1555	206,091	78,723	39,432	61,294	8574	22,519	7260	904
Quercus	0	0	0	44	0	0	0	0	0	0	130	538	0	0	0	0	121	56	0
Rhus	62	0	0	0	103	0	0	0	0	0	43	0	0	0	0	0	0	0	0
Ulmus	0	0	0	44	0	0	0	0	0	0	0	0	0	0	0	0	0	0	90
Yucca	0	0	0	0	0	0	1654	0	0	0	0	0	0	0	0	0	0	0	0

The taxa are listed by laboratory number for each coprolite. Seventeen taxa could be identified to the genus level. The others could be identified only to the family level and are abbreviated in the table: Aster. H.S. = high spine Asteraceae, Aster. L.S. = low spine Asteraceae, Brassicac. = Brassicaceae, Cheno-Am = Chenopodiaceae and/or Amaranthaceae, Euphorb. = Euphorbiaceae.

Table 2

Comparison of pollen types Hinds Cave coprolites that have a ambient source in the lower Pecos region

	Early Archaic n = 7 Dean (2006)	Middle Archaic this study n = 19	Late Archaic n = 50 Edwards (1990)
Alnus	X	X	X
Asteraceae, *Artemisia*	X		X
Asteraceae, high spine	X	X	X
Asteraceae, low spine	X	X	X
Carya		X	
Celtis	X	X	X
Cheno/Am	X	X	X
Ephedra	X	X	X
Fraxinus		X	X
Juglans	X	X	X
Juniperus	X		
Pinus	X	X	X
Poaceae	X	X	X
Quercus	X	X	X
Salix			X
Sarcobatus			X
Typha			X
Ulmus	X	X	X

animals to species level is very difficult in coprolite analysis and that genus identification is more valid. Dean detailed the small animal consumption habits of Early Archaic Hinds Cave dwellers (Williams-Dean, 1978). The food habits of these mammals are summarized by Davis (1974). The following data from these sources. Wood rat bones (*Neotoma*) were found in 19% of Hinds Cave coprolites. Eating whole wood-rats would introduce cpDNA into human digestive systems because in the area of Hinds Cave, various woodrat species eat cactus, acorns, sotol (*Dasylirion*), *Agave*, and mesquite. Cotton rat (*Sigmodon*) bones were found in 13% of the coprolites. They eat grasses, sedges, and herbs. *Peromyscus*, which includes many species of small mouse, was found in 2% of the coprolites. *Peromyscus* species in the area eat seeds, insects,

Table 3

Q2 The most likely plant origins as represented by the best hits defined by maximal identity of a BLAST search of the *rbcL* gene sequence of three taxa recovered by Poinar et al. (2001) are presented in column 5 under the heading Genus

Sequence family identified	GenBank similarity (%)	Subfamily	GenBank similarity (%)	Genus	GenBank similarity (%)
Rhamnaceae	98−100	Rhamneae	98−100	*Rhamnus*	99−100
				Rhamanidium	100
				Sageretia	100
Solanaceae	-100	Solanoidae		*Solanum*	99−100
				Lycopersicon	100
Fouquieriaceae	-100			*Fouquieria*	100

For Rhamnaceae, *Rhamnus* and *Sageretia* are the most likely sources of the cpDNA. *Rhamanidium* is not endemic to the region. For Solanaceae, the *rbcL* gene sequence of the cultivated tomato is the best match. Certainly cultivated tomato could not be the source of the cpDNA. It is conceivable that this cpDNA has an origin in native, wild species of *Solanum*. Importantly, the genera suggest for Solanaceae by Poinar et al. (2001) *Nicotiana*, *Physalis*, *Lycium*, and *Datura* do not match the recovered Solanaceae cpDNA. Finally, *Fouquieria* is a perfect match for the Fouquieriaceae cpDNA.

hackberry (*Celtis*), acorns, and mesquite. Ground Squirrel (*Spermophilus*) bones were found in 3% of the coprolites. In the Hinds Cave region, *Spermophilus* species eat sunflower (*Helianthus*), cactus, mesquite, acorns, pine nuts, walnuts, saltbush, *Agave*, wild gourd, cherries, sumac, spurge, serviceberry, currant berries, and juniper berries. The consumption of herbivorous rodents, with their digestive tracts, would undoubtedly introduce cpDNA into human coprolites.

Application of pollen concentration by previous researchers shows that ambient pollen is found in high amounts in southwestern coprolites (Dean, 2006; Edwards, 1990; Reinhard et al., 1991, 2002, 2006a, b; Sobolik, 1988). This is supported by our results in Table 1. With regard to pollen as a potential source of chloroplast sequences, cpDNA is generally inherited maternally. Therefore, cpDNA should be absent in sperm cells of most plant species. However, cpDNA can be present in DNA-containing proplastids, especially in the vegetative cells within pollen (Bennett and Parducci, 2006; Pacini et al., 1992; Sangwan and Sangwan-Norreel, 1987; Schmitz and Kowallik, 1987; Sodmergen et al., 1994). Therefore, DNA consistent with cpDNA might be present in pollen grains. As more analysis of pollen grains for cpDNA are published, it becomes apparent that we can not exclude inhaled pollen as a source of cpDNA in coprolites. For example, Paffetti et al. (2007) report an estimated 1000 cpDNA molecules per *Fagus* pollen grain. *Fagus* is a close relative of *Quercus* (oak) and one genus in the Fagaceae family represented by cpDNA sequences in Poinar et al. (2001) study. There is no published study on the cpDNA content of *Quercus* pollen. Our analysis shows and average of 47 *Quercus* pollen grains per gram of coprolite. If *Quercus*, like *Fagus*, contains cpDNA in pollen grains, then even small numbers of inhaled pollen could introduce many thousand cpDNA molecules into coprolites.

More importantly, pollen signals the presence of ambient plant material that can be included in drinking water. Thus, accidental ingestion of pollen-associated plant structures might introduce cpDNA into the human digestive tract. For example, *Quercus* disseminates pollen from catkins which are structures that fragment and fall from the source tree. Thus, *Quercus* pollen and photosynthetic plant cells are ambient plant residues from the same source. If pollen grains were ingested accidentally with other plant microresidues such as catkin, flower, florette, leaf or stem fragments, cpDNA could be detected in molecular analysis of coprolites. We suggest that the ingestion of ambient plant residue can be signaled both by cpDNA sequences and pollen in coprolites.

Table 4 compares the list of cpDNA sequences recovered by Poinar et al. (2001) with the percentage of coprolites that contain ambient pollen from the taxa represented by the cpDNA found in this study. This information is compared with the food habits of Hinds Cave prey animals and review of dietary use of these plants in previous coprolite studies from the lower Pecos (Reinhard, 1992). Asteraceae cpDNA sequences were found in the three coprolites analyzed by Poinar et al. (2001), but these coprolites contained no Asteraceae macrofossils. Asteraceae pollen is present in all 19 coprolites and in all Archaic periods. Asteraceae genera are eaten by the prey

Table 4

To interpret the cultural versus ambient source cpDNA found in molecular analysis (Poinar et al., 2001), pollen signals of ambient plant residue and the dietary habits of lower Pecos animals that were eaten by prehistoric hunter-gathers must be considered

Taxa found by Poinar et al. (2001)	Genera suggested by Poinar et al. (2001)	% of coprolites w/pollen contamination	Genera eaten by prey animals? [8]	Genera found in previous studies [16]
Asterales/ Asteraceae	*Helianthus* and many other genera	100%	Yes	*Helianthus*
Ericales/ Fouquieriaceae	*Foquieria*	0%	No	None
Fabales/ Fabaceae	*Acacia, Prosopis, Sophora, Mimosa*	11%	Yes	*Prosopis, Acacia*
Fagales/ Fagaceae	*Quercus*	26%	Yes	None
Liliales/ Liliaceae	*Allium, Nolina Dasylirion, Yucca*	74%	Yes	*Allium, Dasylirion*
Rhamnales/ Rhamnaceae	*Colubrina, Condalia Karwinskia*	0%	No	None
Rosales/ Ulmaceae	*Celtis*	53%	Yes	*Celtis*
Solanales/ Solanaceae	*Nicotiana, Physalis, Lycium,* or *Datura*	0%	No	None

Columns 1 and 2 present the cpDNA discoveries and interpretation. Column 3, based on pollen analysis of 19 coprolites, presents the percentage of coprolites containing plant residue signaled by pollen in coprolites. Fourth columns notes if the taxon represented by cpDNA is a known food habits of animals eaten by Hinds Cave inhabitants [8]. The final column list general in the taxa represented by cpDNA that are known to be Hinds Cave foods [16].

animals consumed by Hinds Cave inhabitants. Reinhard (1992) summarizes that sunflower seeds were a common Hinds Cave food for humans. Therefore, the cpDNA sequences could be ambient plant residue from several sources or derived from plants intentionally eaten by Hinds Cave residents.

Fagaceae cpDNA sequences were found in 2 of 3 analyzed coprolites, but Fagaceae macrofossils were not found. Poinar and his colleagues suggested that acorns (*Quercus*) were the source of this cpDNA. In previous studies of Hinds Cave coprolites, acorns are absent in macrofossil and microfossil analysis (Reinhard, 1992). However, *Quercus* pollen was found in five coprolites and in all Archaic time periods. Acorns are eaten by Hinds Cave prey animal species. Oak catkin fragments, signaled by pollen, could have been consumed with drinking water. It is unknown as to whether inhaled oak pollen could be a source of cpDNA, so this source cannot be discounted. Therefore, we believe that there is a high probability that Fagaceae cpDNA sequences came from ambient plant residue.

Poinar et al. (2001) recovered multiple clones of Ulmaceae from all three of their coprolites but Ulmaceae (*Celtis*) seed from only one. As reviewed by Reinhard (1992), *Celtis* was a minor human dietary component for several Archaic periods. Also, *Celtis* is part of the recorded diet for Hinds Cave prey animals. We recovered *Celtis* and/or *Ulmus* pollen from 10 coprolites in our study. Thus, Ulmaceae cpDNA sequences could be from ambient or intentional consumption of Ulmaceae plants.

Fabaceae and Liliaceae sequences were found in coprolites that also had macrofossils of these families (Table 4). They are very common Archaic foods for all periods. Therefore, there can be little doubt that the cpDNA sequences represent dietary use. The fact that we found high concentrations of Liliaceae (*Dasylirion*) pollen in the majority of the coprolites supports the dietary conclusion for cpDNA.

The most interesting cpDNA sequences are from taxa for which there are no previous documented dietary use at Hinds Cave in coprolite analysis, nor evidence in pollen analysis, and for which there is no record of consumption by prey animals. These sequences are Fouquieriaceae, Rhamnaceae, and Solanaceae. They cannot be explained as ambient plant residue, nor can they be considered as dietary in the light of what is known about prehistoric foods from lower Pecos coprolite studies. However, there are genera in each of these taxa that were used as medicines ethnographically in the lower Pecos region. This raises the possibility that cpDNA reflects medicinal or ritual use. It is possible that the cpDNA originated in medicinal preparations of some of these genera.

Comparison of the cpDNA sequences with GenBank data verifies Poinar et al. (2001). The find of *Fouquieria* (ocotillo) cpDNA is very interesting. The bark of this plant is streaked with green, photosynthetic tissue. It is used to make medicinal tea or poultices for a variety of purposes (Powell, 1988). Therefore, the find of cpDNA sequences from *Fouquieria* is consistent with using the bark for medicinal beverages.

For Rhamnaceae, Poinar et al. (2001) suggested *Karwinskia*, *Condalia*, and *Colubrina*. *Karwinskia* and *Culubrina* are toxic. However, the roots and bark of *Condalia* are a source for antibacterial compounds (Powell, 1988). Condalia seeds were found in 3000 year-old deposits in Hinds Cave and the fruits are edible (Dering, 1979). However, our comparison of the cpDNA sequence with GenBank reveals a 100% match with species of *Rhamnus* and *Segeretia*. One endemic *Rhamnus* species is used in the Southwest as a cathartic.

Poinar et al. (2001) suggested that *Nicotiana*, *Physalis*, *Lycium*, and *Datura* are the genera represented by Solanaceae cpDNA sequences. *Lycium* and *Physalis* were prehistoric food plants in parts of the Southwest, but they are not known as foods from Hinds Cave or other Lower Pecos sites (Reinhard, 1992). There is circumstantial, artifact evidence that *Nicotiana* was smoked in the Hinds Cave region in the form of stone pipes (Chandler, 1990, 1992; Chandler & Boyd, 1995). If it was also chewed, then cpDNA would be swallowed. However, there is no published record of *Nicotiana* quids or other *Nicotiana* evidence in the region. *Datura* is a hallucinogenic plant that is associated with rock art in the region (Adovasio and Fry, 1976; Boyd and Dering, 1996). Teas were made from this plant and it is possible that cpDNA was ingested in this way. Poinar et al. (2001) provide data that should stimulate more research into defining the enteric use of these plants.

However, our match of the cpDNA Solanaceae sequence was 100% consistent with cultivated tomato. This is an impossibility since cultivated tomato never existed in the region prehistorically. It is not consistent with the genera suggested by Poinar et al. (2001). Wild species of *Solanum* could

conceivably have the same *rbcL* gene as cultivated tomato. At this point, however, the Solanaceae cpDNA eludes specific interpretation.

5. Conclusions

The implications of cpDNA sequences in Hinds Cave coprolites may be more complicated than the dietary explanation presented by Poinar et al. (2001) but at the same time they are more interesting. We believe that they made the correct dietary interpretation for Fabaceae and Liliaceae. However, the cpDNA sequences for Ulmaceae, Fagaceae, and Asteraceae could be derived whole or in part from ambient plant residue.

The most intriguing discoveries are of cpDNA from Fouquieriaceae, Rhamnaceae, and Solanaceae that have medicinal or hallucinogenic genera. The cpDNA sequences, therefore expand the potential of paleoethnobotany to include the non-dietary use of economic plants. In this case, molecular biology may expand our knowledge of prehistoric plant use to include plants used for drugs that cannot be discovered using conventional coprolite analysis methods. They provide revealing information into plant use beyond the more mundane dietary explanation and touch on paleopharmacology (Reinhard et al., 1991).

The field of metagenomics is expanding. The potentiality of recovering hundreds or thousands of clones from a single coprolite is becoming a reality. These valuable data must be interpreted with an ecological, cultural, and technological understanding of the people who left the coprolites. Obviously, cpDNA sequences can come from dietary use. In addition to diet, medicinal use of plants must be considered. Infusions, i.e. beverages, could be another source of cpDNA clones. Ambient plant residue inside vertebrate and invertebrate foods must be considered as a source of unintentional plant ingestion. For example, the intentional consumption of grasshoppers results in the unintentional consumption of the grasshoppers' foods. Plant residue can enter the intestinal tract from processing plant fibers with teeth. This could be another source of cpDNA clones that was unintentional. Plant residue in water or air, represented by pollen in coprolites, would be a source of ambient environmental cpDNA sequences. Finally, plant residues on tools or even on hands that are left from processing plants for non-dietary purposes could enter the mouth. In short, any plant that is held in the mouth, or passes through the mouth, or that is clean off of tools and skin with the mouth for any reason, could leave trace amounts of cpDNA for metagenomic recovery and interpretation. For this reason, researchers have to develop a clear understanding of the archaeology of the research area to develop and understanding of cultural and environmental sources of cpDNA. At the same time, molecular biology expands greatly what we can see with our microscopes and therefore challenges us to explore the nexus of archaeobotany, ethnobotany, and ecology to optimize the interpretation of molecular data.

Uncited references

Q6 Bennet, 2006; Wellmann, 1978.

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