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A molecular analysis of ground sloth diet through the last glaciation

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Abstract

DNA was extracted from five coprolites, excavated in Gypsum Cave, Nevada and radiocarbon dated to approximately 11 000, 20 000 and 28 500 years BP. All coprolites contained mitochondrial DNA sequences identical to a DNA sequence determined from a bone of the extinct ground sloth *Nothrotheriops shastensis*. A 157-bp fragment of the chloroplast gene for the large subunit of the ribulosebisphosphate carboxylase (*rbcL*) was amplified from the boluses and several hundred clones were sequenced. In addition, the same DNA fragment was sequenced from 99 plant species that occur in the vicinity of Gypsum Cave today. When these were compared to the DNA sequences in GenBank, 69 were correctly (two incorrectly) assigned to taxonomic orders. The plant sequences from the five coprolites as well as from one previously studied coprolite were compared to *rbcL* sequences in GenBank and the contemporary plant species. Thirteen families or orders of plants that formed part of the diet of the Shasta ground sloth could be identified, showing that the ground sloth was feeding on trees as well as herbs and grasses. The plants in the boluses further indicate that the climate 11 000 years BP was dryer than 20 000 and 28 500 years BP. However, the sloths seem to have visited water sources more frequently at 11 000 BP than at earlier times.

Keywords: ancient DNA, climate change, coprolites, diet, sloth, vegetation change

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Introduction

At the end of the last glaciation, some 11 000 years ago, at least 33 genera and more than 50 species of large mammals disappeared from the North American continent (Martin 1984). In addition to bones and rare soft tissue remains, several of these animals left large deposits of dung behind, particularly in caves that they inhabited or visited regularly. These remains are valuable because they offer insights into the diet of these creatures, the behaviour of the animals, and the flora during the late Pleistocene.

Coprolites from the extinct megafauna of the Americas have been studied for over 60 years (Laudermilk & Munz 1934). In particular, several macro- and microscopic studies have been performed on the dung of the extinct ground sloth *Nothrotheriops shastensis* (Hansen 1978; Thompson *et al*. 1980). In addition to a large number of plants that are rare in the coprolites, these studies have identified mormon tea (*Ephedra nevadensis*), globemallow (*Sphaeralcea ambigua*) and saltbushes (*Atriplex* spp.) as major parts of the ground sloth diet. Recently, we showed that coprolites contain DNA that can be released upon the addition of a chemical compound, N-phenacyl thiazolium bromide (PTB), during the extraction procedure (Poinar *et al*. 1998). This compound breaks crosslinks between reducing sugars and primary amines (Vasan *et al*. 1996), formed by the Maillard reaction (Ledl & Schleicher 1992). This reaction has affected a coprolite from Gypsum Cave (Poinar *et al*. 1998) and is likely to have affected many ancient tissues (Pääbo 1990). PTB treatment allowed DNA sequences both from the animal that deposited the coprolite and from the plants it had ingested to be determined. This may greatly improve the understanding of the ecology and behaviour of extinct animals because many plants are

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hard or impossible to identify morphologically after mastication and passage through the gastrointestinal tract. Here we report the molecular analysis of six late Pleistocene coprolites from Gypsum Cave, Nevada (36°12′ N latitude, 114°39′ W longitude, 580 m a.s.l).

Materials and methods

Dating

The five coprolites were radiocarbon dated in the Ångström laboratory, Uppsala, Sweden. Ages and laboratory numbers were: #1, 11 005 ± 100 years BP (Ua-12506); #2, 11 080 ± 90 years BP (Ua-12509); #3, 19 500 ± 205 years BP (Ua-13223); #5, 27 810 ± 455 years BP (Ua-12508); and #6, 29 205 years BP (Ua-13224). The date for #4 is in Poinar *et al*. 1998.

DNA extraction, PCR and sequencing

DNA was extracted (Rogers & Bendich 1985) from 112 herbarium specimens of plants from the Gypsum Cave area (for a list of the plant species see http://www.eva.mpg.de/analysis/index.html). Briefly, 100 mg of plant tissue were placed in an Eppendorf tube together with 500 µL extraction buffer (1% cetyltrimethyl ammonium bromide [CTAB], 10 mM EDTA, 50 mM Tris-HCl (pH 8.0), 0.7 M NaCl, 50 mM 2-mercaptoethanol), shaken for 2 h at 75 °C and chloroform extracted twice. The aqueous phase was transferred to a new tube, 500 µL of precipitation buffer (1% CTAB, 10 mM EDTA, 50 mM Tris-HCl) was added, and the DNA was precipitated by centrifugation at 5700 *g* at room temperature. DNA pellets were resuspended in 100 µL TEN buffer (10 mM Tris-HCl, 1 mM EDTA, 1 M NaCl) and precipitated by addition of 200 µL of 100% ethanol. The DNA was resuspended in 100 µL TE buffer (1 mM Tris-HCl, pH 8.0, 0.1 mM EDTA) and 2 µL were used for 30 cycles of polymerase chain reaction (PCR) with primers *rbcL* Z1aF and hp2R, using an annealing temperature of 55 °C, AmpliTaq (Perkin Elmer, USA) and the reagents recommended by the supplier. Ninety-nine samples yielded amplifiable DNA.

DNA from coprolites was extracted as described (Poinar *et al*. 1998) with minor modifications. Coprolite pieces were ground to a fine powder under liquid nitrogen with a pestle and mortar. To 0.2 g of powder, 1.4 mL of extraction buffer (0.1 M Tris-HCl (pH 8.0), 2 mM EDTA, 0.7 M NaCl, 1% SDS, 50 mM DTT, 0.2 mg/mL proteinase-K) were added. Samples were incubated for 24 h at 37 °C on a rotary shaker and 100 µL of a 100-mM PTB solution were added. The incubation was continued for another 72 h. The samples were extracted twice with chloroform and DNA was recovered by binding to silica (Höss & Pääbo 1993; Poinar *et al*. 1998).

DNA from the *Nothrotheriops shastensis* bone was extracted as follows: 0.2 g of ground bone was incubated at 37 °C for 24 h in 1.4 mL of extraction buffer (0.5 M EDTA, 5% N-lauryl sarcosine, 1% polyvinylpyrolidine, 50 mM DTT and 140 µg/mL proteinase-K). After addition of 150 µL of 100 mM PTB and 150 µL of 8% CTAB the incubation was continued for another 72 h. The sample was centrifuged briefly, the supernatant extracted twice with chloroform:isoamylalcohol (24:1) and DNA recovered by binding to silica as described (Höss & Pääbo 1993; Poinar *et al*. 1998). Finally, the silica pellet was dried for 5 min at 56 °C and eluted with 130 µL TE for 8 min at 56 °C. PCR was performed as described (Höss & Pääbo 1993) using a hot-start protocol.

Primers used were: 16S6 5′-TTTCGGTTGGGGCGACCTCGGAG-3′; 16S7 5′-TTGCGCTGTTATCCCTAGGGTAAC-3′; 16SNS1 5′-CCTCCGAACGACTATGCGCCCA-3′; *rbcL* Z1aF 5′-ATGTCACCACCAACAGAGACTAAAGC-3′; *rbcL* 19bR 5′-CTTCTTCAGGTGGAACTCCAG-3′; and hp2R 5′-CGTCCTTTGTAACGATCAAG-3′.

PCR products for the 16S6–16S7 (Höss *et al*. 1996a) fragments were reamplified with the primer pair 16SNS1 and 16S7 and sequenced directly, using the primer 16S7. The *rbcL* fragments from the coprolites were amplified and reamplified using the primer pair Z1aF (3′ end at position 54 983 in the *Arabidopsis thaliana* chloroplast genome, GenBank NC 000932.1) and 19bR (3′ end at pos. 55 094). The fragments were made blunt-ended by treatment with T4-DNA polymerase and cloned in pUC18 (Amersham Pharmacia Biotech, Germany). For each specimen, three independent amplifications of the *rbcL* fragment were performed, and the sequences of 73 or more clones were determined.

PCR products from herbarium specimen were sequenced directly, using the primer hp2R (3′ end at position 55 186), the 'Thermo Sequenase Kit' (Amersham Pharmacia Biotech, Germany) and an ALF Sequencer (Amersham Pharmacia Biotech, Germany).

DNA sequence analysis

Sequences of the *rbcL* fragment were aligned and grouped into clusters by eye. All unique sequences were compared with the approximately 3600 *rbcL* sequences in GenBank by means of the BLAST program (Altschul *et al*. 1997) and family(ies) and order(s) of the closest matches were noted. Consensus sequences were also compared to the sequences determined from herbarium specimens. All herbarium sequences were compared to sequences in GenBank by means of the BLAST program, and the number of mismatches to the most similar sequence in the database were noted. In two cases, the length of the GenBank sequences showing the highest similarity to the fragment determined was only 108 bp, and in 11 cases it was 106 bp. In these cases, comparisons were limited to these lengths.

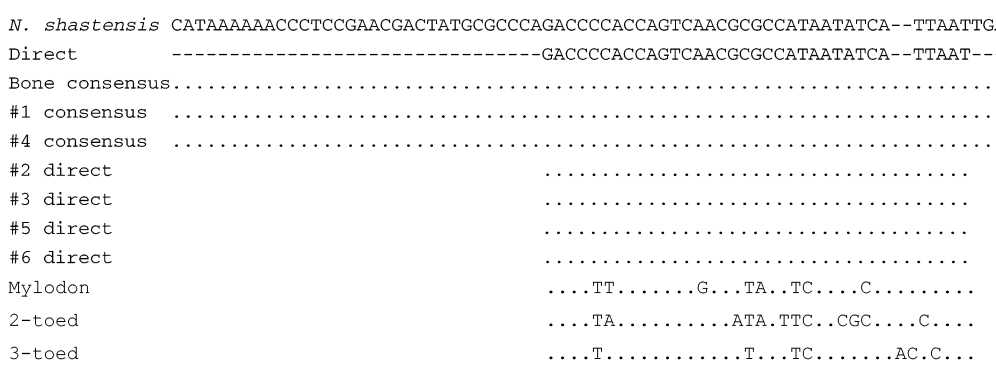

Fig. 1 Alignment of the 16S rDNA sequences from six Pleistocene coprolites and a *Nothrotheriops shastensis* bone. The PCR products from boluses 2, 3, 5, and 6 were sequenced directly, while the products from boluses 1 and 4 and from the bone were cloned and five or more clones sequenced. In the latter cases, the consensus sequences are shown. In addition, the closest matches found in GenBank (two- and three-toed sloths, Mylodon ground sloth; accession nos Z48941, Z48937 and Z48943, respectively) are shown.

Results

Dating and identification of defaecating species

Samples from six intact coprolite boluses from Gypsum Cave were dated by tandem accelerator mass spectrometry and five samples were selected such that, together with one bolus previously analysed (Poinar *et al*. 1998), they represent three time points that span the Middle and Late Wisconsinan glaciation. The two oldest boluses were around 28 500 years old, a period of increased precipitation and relatively mild temperatures in the American South-west. The two middle ones were around 20 000 years old, close to the peak of the last glaciation around 18 000 BP (Spaulding & Graumlich 1986; Quade *et al*. 1995). Finally, two coprolites were close to the time of extinction of the Shasta ground sloth about 11 000 years BP (Long & Martin 1974), at the end of the last glacial period.

DNA was extracted from the five coprolites in the presence of the compound PTB which breaks crosslinks formed by the Maillard reaction. To identify the species that produced the boluses, a 142-bp fragment of the mitochondrial 16S ribosomal RNA gene was amplified from the extracts as well as the extract of the coprolite previously described from Gypsum Cave (Poinar *et al*. 1998). All amplifications yielded products that could be visualized in ethidium-stained agarose gels. The bands were isolated from the gels and in two cases (#1, 4) reamplified, cloned and the sequences of multiple clones determined. In the remaining four cases the products were sequenced directly using an internal primer that allowed 35 bp to be determined. All six sequences were identical (Fig. 1).

From the coprolite previsously described, a 12S rDNA sequence had shown it to derive from a sloth (Poinar *et al*. 1998), presumably *Nothrotheriops shastensis*, the ground sloth from which bones have been found in the cave. However, to ascertain that the boluses are indeed derived from this sloth species, the 142 bp piece of the 16S rDNA was amplified and sequenced from a bone morphologically determined to be *N. shastensis* from Rampart Cave, Arizona. This sequence was found to be identical to the coprolite sequences while carrying five substitutional differences and two deletions to the closest DNA sequence present in GenBank, the three-toed sloth (*Bradypus variegatus*, Fig. 1). Thus, the coprolites found in the cave can be positively associated with the species *N. shastensis*.

Pleistocene rbcL *DNA sequences*

To investigate the diet of the sloth, a 157-bp fragment of the large subunit of the ribulose-bisphosphate carboxylase (*rbcL*) gene, located in the chloroplast genome, was amplified (Fig. 2). For each bolus, three independent amplifications were performed, amplification products were cloned and clones were sequenced until 15 consecutive clones failed to lead to the identification of any new plant taxa according to the procedure described below. Out of the 514 clones sequenced from the five coprolites, 496 could be easily organized into 40 groups or 'clusters' of related sequences, including five clusters consisting of single sequences. The nucleotide sequence variation within the clusters was interpreted to be due to nucleotide misincorporation during the PCR, a common phenomenon when amplifications are performed from ancient DNA which is highly modified (Höss *et al*. 1996b). For each cluster a consensus sequence was derived. This sequence was interpreted as representing the *rbcL* sequence present in the ancient plant (Handt *et al*. 1996; Poinar *et al*. 1998). When the results from the five coprolites as well as the previously studied coprolite were pooled, 37

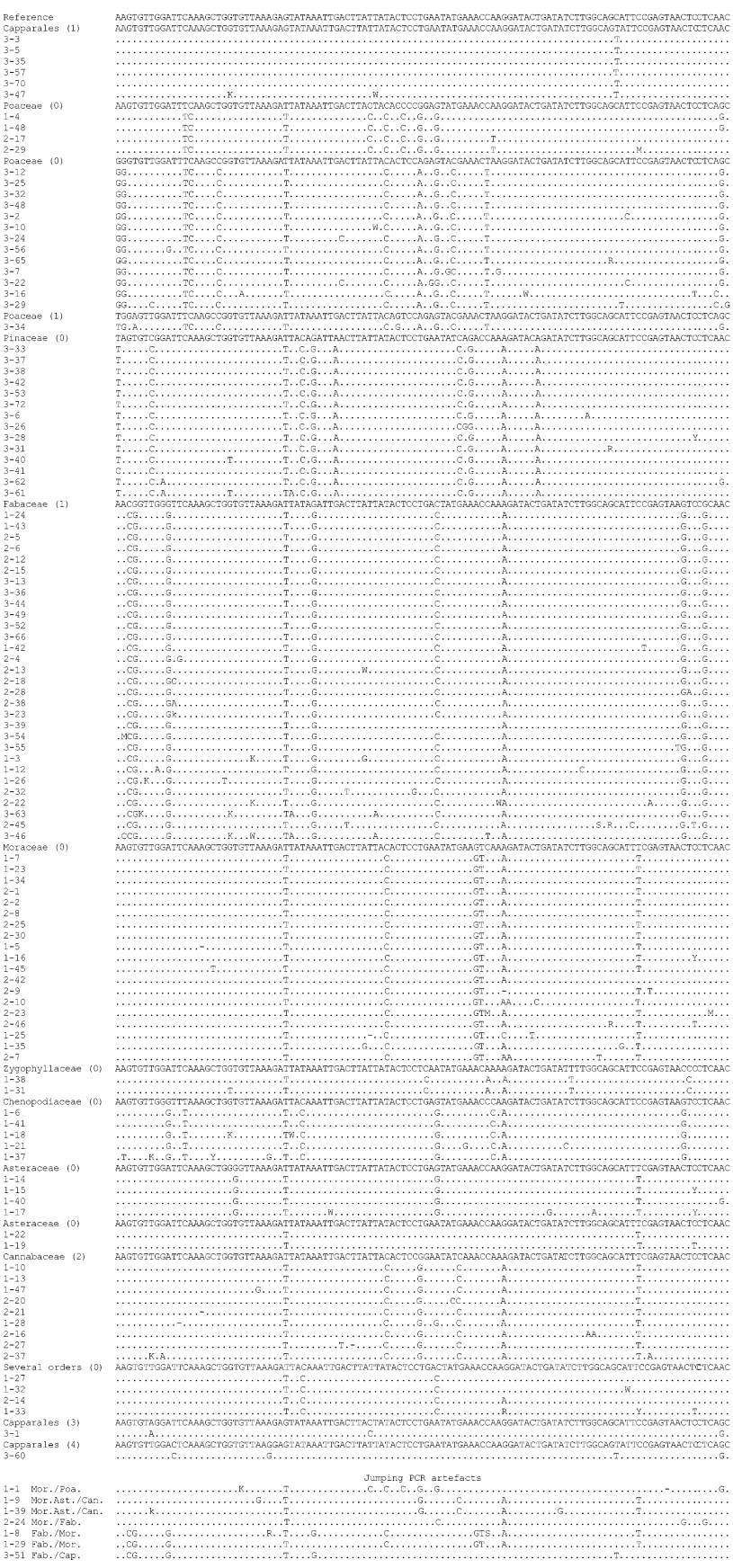

Fig. 2 *rbcL* DNA sequences from bolus 6. Dots indicate identities to the reference sequence (the most common sequence from #4; Poinar *et al*. 1998). Consensus sequences are given above each cluster. Numbers in front of each clone give the extraction and clone number, the numbers in front of the families/orders refer to the numbers in Table 1, while the numbers in parentheses behind the families/orders give the number of differences from the closest GenBank or herbarium sequence. Sequences that seem to represent jumping PCR events are indicated below. The sequences for the other coprolites can be found in Poinar *et al*. 1998 (#4) and at http://www.eva.mpg.de/analysis/index.html.

different sequence clusters and individual sequences were found and used for taxonomic identification (see below).

Eighteen out of the 514 clones sequenced could not be assigned to any cluster of clones because they carried one segment that matched one cluster and another segment that matched another cluster. Such 'jumping-PCR' effects have been previously observed when PCR is performed from ancient DNA (Pääbo *et al*. 1990; Poinar *et al*. 1998). They are the result of damage as well as strand breaks in the template molecules that cause premature termination of the primer extensions. In subsequent cycles of the PCR, such products can be extended on other template molecules resulting in recombinant amplification products. Most 'jumping-PCR' artefacts were observed in the coprolites where large numbers of different plant sequences were found, probably because jumping events can be identified only when they occur between molecules of different sequence. All sequences interpreted as jumping artefacts were excluded from further analysis.

Contemporary rbcL *DNA sequences*

In order to increase the number of *rbcL* DNA sequences relevant for the identification of the ancient plant sequences from Gypsum Cave, we amplified and sequenced the *rbcL* fragment from 99 herbarium specimens of contemporaneous plant genera found in the vicinity of Gypsum Cave. Most of these herbarium specimens have been collected in Nevada and may, therefore, be better suited for identification of Gypsum Cave plants than GenBank sequences, which are derived from a much larger geographical area.

Plant identification

To assess the efficiency with which the taxonomic affiliation of *rbcL* DNA sequences can be determined, the herbarium sequences, for which the correct taxonomic affiliations were known, were compared to the 3600 *rbcL* sequences deposited in GenBank by means of the BLAST program. In each case, the family(ies) and order(s) that differed at 0, 1, and 2 nucleotide positions from the herbarium sequences were noted.

An identification was classified as 'correct' only when the correct family or order of plants was found, as 'ambiguous' when several families/orders were found, and as 'incorrect' when only one, but incorrect family/order was found. It was also noted when no taxon in GenBank matched the sequence at two or less differences (Fig. 3). At the order level and zero differences, the identification was correct in 69 of the 99 herbarium specimen, ambiguous in two specimens, incorrect in two specimens, and failed in 26 specimens due to no matches in the database. At the family level, identifications were correct in 47 cases, ambiguous in 23 cases, incorrect in three cases and failed in 26 cases. At one difference, the identification of the order is correct and ambiguous in approximately equal number of cases, whereas at two differences there are more ambiguous than correct identifications. At the family level, the identification is ambiguous in the majority of the cases at both one and two differences (see http://www.eva.mpg.de/analysis/index.html for a full list of the BLAST results). It is noteworthy, that for 26 taxa, no identical sequences are found in GenBank. This number is expected to decrease as the number of *rbcL* sequences increases.

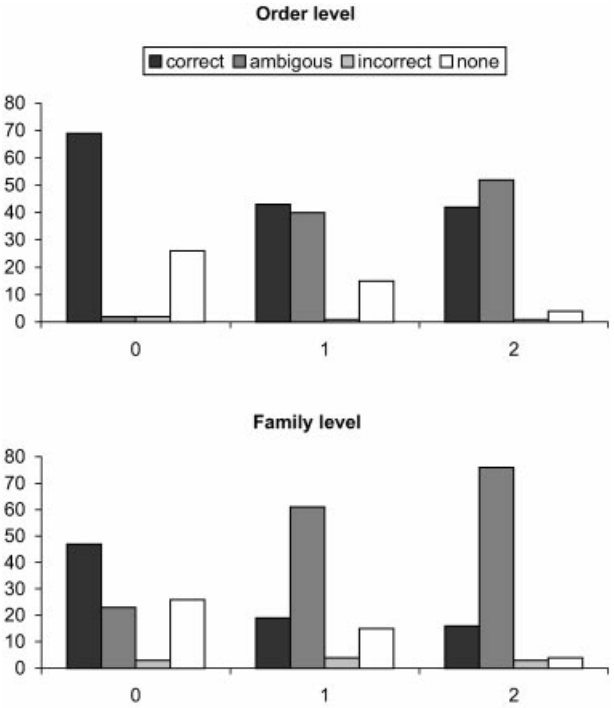

Fig. 3 Taxonomic identification of 99 herbarium DNA sequences. The number of sequences for which the identifications at the order and the family levels were correct, ambiguous, incorrect or no match was found are given for 0, 1, and 2 mismatches, respectively.

Identification of Pleistocene plants

For the identification of the ancient *rbcL* sequences, only identical sequences in GenBank or among the herbarium sequences were used. The only exception was one sequence cluster from bolus #5, where the consensus sequence carried a stop codon at positions 95–97, which was interpreted as a PCR error. Based on molecular evidence alone, only identification at the order level was performed, although it was noted when only one or two families or genera were identified (Table 1).

Table 1 Taxonomic identification for 21 consensus sequences found in the six coprolites

			GenBank			Herbarium		
Cons. seq.	bolus #	age 1000 years	order	families	genera	order	families	genera
1	1, 4	11; 20	Capparales	3	8	Capparales	Brassicaceae	3
							Capparaceae	2
2	1, 4	11; 20	Asparagales	13	33	Asparagales	Liliaceae	Yucca
			Liliales	2	4			Nolina
3	1, 2	11	Lamiales	10	41	Lamiales	3	3
			Gentianales	1	2			
4	1, 2	11	Caryophyllales	Chenopodiaceae	Atriplex	Caryophyllales	Chenopodiaceae	Atriplex
5	1	11	Asterales	Asteraceae	Lactuca			
6	1	11				Asterales	Asteraceae	Brickellia
7	1, 6	11; 28.5	Asterales	Asteraceae	5	Asterales	Asteraceae	8
8	1	11	Rosales	Rosaceae	7			
9	1, 2	11	Rhamnales	Vitaceae	Vitis	not in herbar.		
10	2	11	Malpighiales	Salicaceae	Salix	Malpighiales	Salicaceae	Salix
					Populus			
11	3, 5, 6	20; 28.5	Rosales	Moraceae	Morus	not in herbar.		
12	3, 6	20; 28.5	Poales	Poaceae	Stipa			
13	5, 6	28.5	Coniferales	Pinaceae	Pinus	not in herbar.		
14	5	28.5	Lamiales	Lamiaceae	4			
15	5	28.5	Capparales	Brassicaceae	Brassica			
16	6	28.5	Poales	Poaceae	Plinthanthesis			
					Karroochloa			
17	6	28.5	Zygophyllales	Zygophyllaceae	Larrea	Zygophyllales	Zygophyllaceae	Larrea
18	6	28.5				Caryophyllales	Chenopodiaceae	Grayia
19	6	28.5	Asterales	Asteraceae	Achillea	Asterales	Asteraceae	Artemisia
20	4	20	Poales	Poaceae				
21	4	20	Euphorbiales	Euphorbiaceae				

Numbers in the columns 'families' and 'genera' indicate how many families/genera were identical to the consensus sequence. Consensus sequence numbers correspond to numbers in Fig. 2 and the web page information. Sequences 20 and 21 are from Poinar *et al*. 1998.

Based on the above criteria, 21 of the 37 sequences found in the six coprolites were found to belong to 13 families or orders (Table 2). In several coprolites, sequences identical to different species within the same family/order were found. In three cases where a cluster contained only two sequences, one sequence was identical to a single family, whereas the second sequence had one difference to the same family. In one cluster containing four sequences, two nucleotide differences occurred in two clones each, such that no consensus sequence could be derived (cluster 12 in Fig. 2). Because one difference created a stop codon, this position was disregarded, which allowed the cluster to be assigned to the family Poaceae. By combining the molecular identification with information about the current and past vegetation in the American Southwest, genera could be tentatively assigned to several of the DNA sequences (Table 1).

At 28 500 years BP (Fig. 4), the most common DNA sequences found in the coprolites are from the family Pinaceae (probably the genus *Pinus*, pines), the family Moraceae (probably Texas mulberry, *Morus microphylla*), the order Capparales (capers and mustards) and the family Poaceae (grasses). In addition to these major taxa, the families/orders Lamiales (scrophs and mints), Asteraceae (asters), Chenopodiaceae (hop sage, genus *Grayia*) and Zygophyllaceae (creosote bush, genus *Larrea*) were identified, each from six or less clones.

At 20 000 years BP, Capparales (capers and mustards) and Liliales (lilies, genus *Yucca* or *Agave*) are the most commonly identified orders. The family Poaceae (grasses), although not very common, is found in both coprolites. Other families/orders identified are Moraceae (Texas mulberry, *Morus microphylla*) and Euphorbiaceae (milkweeds).

At 11 000 years BP, the most common family is Chenopodiaceae (saltbushes, genus *Atriplex*), followed by the order Lamiales (scrophs and mints), and the families Asteraceae (asters) and Vitaceae (grapes, genus *Vitis*). Further families/orders are Liliales (lilies), Capparales (capers and mustards), Salicaceae (genus *Salix* or *Populus*) and Rosaceae (roses).

Table 2. Number of clones assigned to an order/family

	Average age	11 000		20 000		28 500	
	Bolus number	1	2	3	4	5	6
Order							
Capparales		4			19	31	
Lamiales/ Gentianales		15	36			6	
Caryophyllales (Chenopodiaceae)		20	49				5
Asterales(Asteraceae)		21					6
Rhamnales (Vitaceae)		2	17				
Poales (Poaceae)				6	3		17
Rosales(Moraceae)				8		13	19
Coniferales (Pinaceae)						23	14
Liliales		10			18		
Rosales (Rosaceae)		3					
Euphorbiales (Euphorbiaceae)					1		
Zygophyllales (Zygophyllaceae)							2
Malpighiales (Salicaceae)			4				
unidentified		8	9	96	28		52
jumps		9		2	3		7
Clones sequenced		**92**	**115**	**112**	**72**	**73**	**122**

For bolus #4, only the clones in Poinar *et al*. 1998 representing the same *rbcL* fragment as analysed here are included.

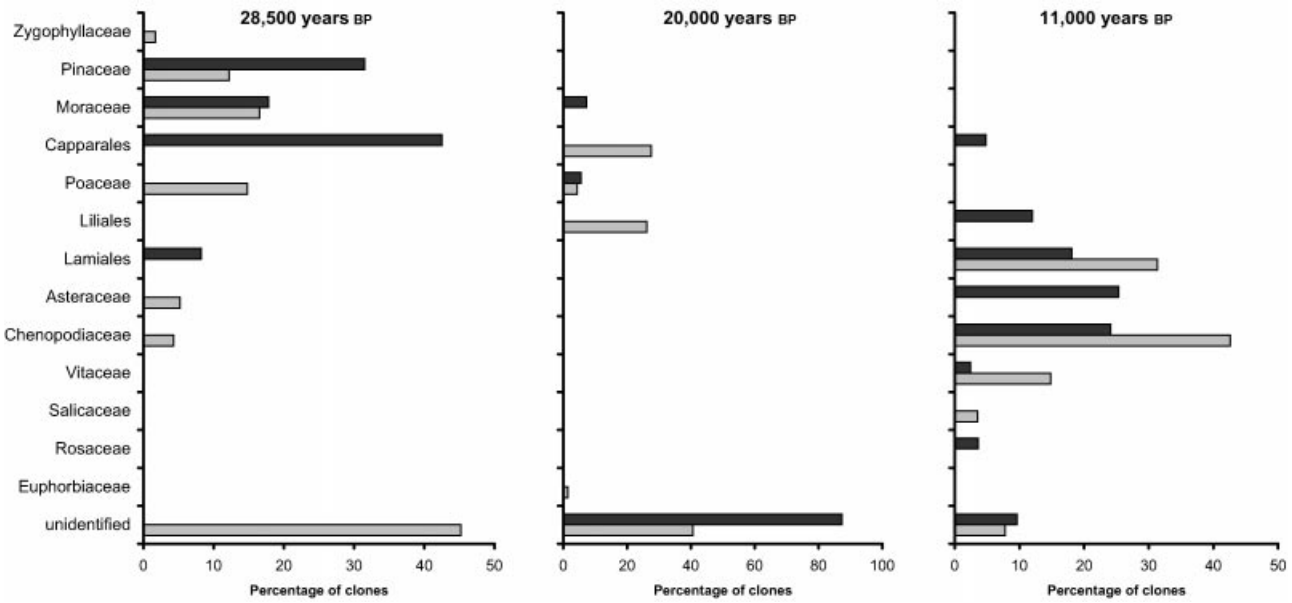

Fig. 4 Plant families and orders identified at the three time points. The *x*-axis gives the percentage of total clones for a certain time point (excluding clones that represent 'jumping PCR' artefacts). The darker bars represent coprolites #5, #3, and #1 (from left to right) and the lighter bars #6, #4 (Poinar *et al*. 1998), and #2, respectively.

Discussion

Technical considerations

The molecular analysis of coprolites opens a new window into late Pleistocene biota. First, it allows mitochondrial DNA sequences from the species that has produced the coprolites to be determined. This makes it possible to identify the defaecator, particularly when homologous DNA sequences can be retrieved from skeletal remains, as was possible here. Moreover, population genetic studies of Pleistocene fauna should become possible

using coprolites. Second, the analysis of coprolites allows plants that formed part of the diet of herbivores to be identified.

The molecular analysis presented here yields a more diverse picture of *Nothrotheriops'* diet than previous histological studies of coprolites from Rampart Cave, Arizona (Hansen 1978), Shelter Cave, New Mexico (Thompson *et al*. 1980) and Gypsum Cave (Laudermilk & Munz 1934). This is because molecular analyses are able to detect plants that may be so rare in the dung, or so modified by masticatory and digestive processes, that they are not easily identified morphologically. However, several aspects of the molecular study of coprolites need further development. For example, it is possible that the extraction procedure selects for plants, or parts of plants (e.g. woody parts), that preserve the DNA better than others. Thus, it will be important to investigate faecal samples from contemporary captive animals where the exact composition of the diet is known to clarify to what extent the abundance of clones reflect the abundance of plants ingested by an animal.

There is no doubt that some of the intersample variability in our data is due to seasonal changes in diet, as well as to the vagaries of foraging routes selected by the individual ground sloths. This is obvious from the observation that a plant frequently present in one bolus from a particular time point can be absent from the other bolus. Thus, in order to determine that a particular plant was absent from the diet at a certain time, much larger numbers of contemporaneous boluses must be studied.

Because the length of the *rbcL* sequences that can be routinely amplified from the coprolites is limited to less than 200 bp, the precision of the identification of sequences found in the coprolites is limited. In most cases, identification based on the molecular data is possible only at the order level. However, if additional information such as the current flora and the palaeobotanical record is taken into account, a more precise putative identification can often be achieved. We expect that as the *rbcL* databases increase in size, the identification efficiency will improve because the number of sequences lacking a match in the database will decrease. Nevertheless, it is encouraging that, based on the 157 bp *rbcL* fragment, the classification of 99 plant specimens was incorrect for only two specimens at the order level, and three at the family level.

Palaeoclimatic and behavioural implications

The time period from 29 000 to 11 000 BP spanned by the coprolites studied include the last glacial interstade and the last glacial stade (the Middle and Late Wisconsinan, respectively). The late interstade enjoyed increased precipitation and relatively mild temperatures, followed by cold and dry conditions during the glacial maximum (≈18 000 BP), followed again by warming temperatures and increased precipitation between 15 000 and 11 000 BP (Spaulding & Graumlich 1986; Quade *et al*. 1995; Spaulding 1995). The molecular analysis of the six coprolite samples yield insights into the flora of the Gypsum Cave area during this time period, as well as the diet of the Shasta ground sloth (Fig. 4).

At 28 500 years BP, the two coprolites show a wide array of plants. Most striking is the presence of Pinaceae, likely originating from the pine *Pinus monophylla*, in both coprolites. This xerophytic tree presently grows on substrate similar to that around Gypsum Cave but is restricted to elevations above ~1800 m. It rarely appears in the full glacial-age palaeoecological record. For example, it has been recorded in only two of 31 Wisconsinan packrat midden samples below 1000 m elevation in this part of the Mojave desert (Van Devender & Spaulding 1979). Of interest is the presence of Moraceae (mulberry), most likely *Morus microphylla* (Texas mulberry), a tree that is presently relictual in northern Arizona. Although the presence of *Pinus* (pines), *M. microphylla* (Texas mulberry) and *Artemisia* (sagebrush) suggests a wetter and colder climate than today, typical desert species are found as well. For example, the genera *Grayia* (hop-sage) and *Larrea* (creosote bush) are common desert scrubs found in the Mojave Desert today (Cole & Webb 1985). The presence of *Larrea* at 28 500 BP is particularly noteworthy because the packrat midden record gives no firm evidence for its presence in Nevada until after ≈8500 years BP (Spaulding 1995). The other families/orders found at 28 500 BP [Capparales (mustards and cappers), Poaceae (grasses), and Lamiales (scrophs and mints and related orders)] are all represented by contemporary genera in the American Southwest.

The samples from 20 000 BP are of particular interest because *Nothrotheriops* is missing from other sites during this period (Spaulding 1990). The most frequent taxa at this time are the orders Capparales and Liliales. The former taxon is represented at both other time points and is thus likely to have been a major element of the *Nothrotheriops'* diet. Of the four families within the Capparales (Capparaceae, Koeberliniaceae, Brassicaceae, and Resedaceae), member(s) of the mustard family (Brassicaceae) are the most likely source of the DNA sequences on biogeographic and palaeoecological grounds. Moraceae (*M. microphylla*, Texas mulberry) and Poaceae (grasses) are both found at 20 000 BP as well as at 28 500 BP. However, two notable differences between these two time points are that *Pinus* (pines, Pinaceae) is missing whereas Liliales (likely *Yucca* and *Agave*; Poinar *et al*. 1998) is present at 20 000 BP.

In contrast to the other samples, the boluses from 11 000 BP are dominated by the Lamiales (and related

orders) and Chenopodiaceae (saltbushes, *Atriplex* spp.), with one of the samples also yielding substantial amounts of Asteraceae. Saltbushes (*Atriplex* spp.) are xerophytic, shrubby members of the Chenopodiaceae common in the vicinity of Gypsum Cave today. Their relative abundance in these samples may reflect vegetational changes (and consequent dietary shifts of the *Nothotheriops*) in response to warming temperatures at the end of the last glacial period. The same is true for the Liliales (*Yucca* and *Agave*) and Rosaceae, which, although absent from the area today, have been consumed by the Gypsum Cave ground sloths. At first glance, the presence of wild grape (Vitaceae) and willow or cottonwood (Salicaceae), which require perennial water for survival, appears hard to reconcile with that of xerophytic plants in the coprolites at 11 000 BP. However, it is possible that the sloths visited water sources more frequently, perhaps in response to increasing temperatures, during the deglaciation. The alternative explanation, that water sources were more common, receives no support from the palaeohydrological record which show little evidence for spring discharge during this cold and dry period (Spaulding 1995). Furthermore, this cannot account for the difference to the 28 500-year-old samples when there was a marked episode of spring discharge (the 'Unit D episode', Quade *et al*. 1995) and yet no evidence of plants requiring perennial water in the sloth diet. Although further sampling is clearly required, we favour the idea that, like large desert herbivores of the present time, frequency of ground sloth trips to water was directly proportional to effective temperature which was higher at 11 000 BP than previously during the Wisconsinan (Spaulding & Graumlich 1986). The closest source of perennial water at this time would have been Las Vegas Wash, about 10 km to the south of Gypsum Cave (Poinar *et al*. 1998). Thus, the feeding range of ground sloths must have been quite extensive, at least towards the end of their existence.

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References

Altschul SF, Madden TL, Schäffer AA *et al.* (1997) Gapped BLAST and PSI BLAST: a new generation of protein database search programs. *Nucleic Acids Research*, **25**, 3389–3402.

Cole KL, Webb RH (1985) Late Holocene changes in Greenwater Valley, Mojave Desert, California. *Quaternary Research*, **23**, 227–235.

Handt O, Krings M, Ward RH, Pääbo S (1996) The Retrieval of Ancient Human DNA Sequences. *American Journal of Human Genetics*, **59**, 368–376.

Hansen RM (1978) Shasta ground sloth food habits, Rampart Cave, Arizona. *Paleobiology*, **4** (3), 302–319.

Höss M, Pääbo S (1993) DNA extraction from Pleistocene bones by a silica-based purification method. *Nucleic Acids Research*, **21**, 3913–3914.

Höss M, Dilling A, Currant A, Pääbo S (1996a) Molecular phylogeny of the extinct ground sloth *Mylodon darwinii*. *Proceedings of the National Academy of Sciences of the USA*, **93**, 181–185.

Höss M, Jaruga P, Zastawny TH, Dizdaroglu M, Pääbo S (1996b) DNA damage and DNA sequence retrieval from ancient tissues. *Nucleic Acids Research*, **24**, 1304–1307.

Laudermilk JD, Munz PA (1934) Plants in the dung of *Nothrotherium* from Gypsum Cave, Nevada. *Carnegie Institute. Washington Publishers*, **453**, 31–37.

Ledl F, Schleicher E (1992) Die Maillard Reaktion in Lebensmitteln und im menschlichen Körper — neue Ergebnisse zur Chemie, Biochemie und Medizin. *Angewandte Chemie*, **102**, 597–626.

Long A, Martin PS (1974) Death of American Ground Sloth. *Science*, **186**, 638–640.

Martin PS (1984) Prehistoric overkill: The global model. In: *Quarternary Extinction: a Prehistoric Revolution* (eds Martin PS, Klein RG), pp. 354–403 University of Arizona Press, Tucson.

Pääbo S (1990) Amplifying ancient DNA. In: *PCR-Protocols and Applications — a Laboratory Manual* (eds Innis MA, Gelfand DH, Sninsky JJ, White TJ), pp. 159–166, Academic Press, San Diego.

Pääbo S, Irwin DM, Wilson AC (1990) DNA damage promotes jumping between templates during enzymatic amplification. *Journal of Biological Chemistry*, **265**, 4718–4721.

Poinar HN, Hofreiter M, Spaulding WG *et al.* (1998) Molecular coproscopy: Dung and diet of the extinct ground sloth *Nothrotheriops shastensis*. *Science*, **281**, 402–406.

Quade J, Mifflin MD, Pratt WL, McCoy W, Burckle L (1995) Fossil spring deposits in the southern Great Basin and their implications for changes in water-table levels near Yucca Mountain, Nevada, during Quaternary time. *Geological Society of America Bulletin*, **107**, 213–230.

Rogers SO, Bendich AJ (1985) Extraction of DNA from milligram amounts of fresh, herbarium and mummified plant tissues. *Plant Molecular Biology*, **5**, 69–76.

Spaulding WG (1990) Vegetational and climatic development of the Mojave Desert: The last glacial maximum to the present. In: *Packrat middens: the Last 40 000 Years of Biotic Change* (eds Betancourt JL, Martin PS, Van Devender TR), p. 199. University of Arizona Press, Tucson.

Spaulding WG (1995) Environmental change, ecosystem responses, and the Late Quaternary development of the Mojave Desert. In: *Quaternary Environments and Deep History: A Tribute Paul S Martin* (eds Mead JI, Steadman DS), pp. 139–164. Frenske Printing, Inc., Rapid City, S.D.

Spaulding WG, Graumlich LJ (1986) The last pluvial climatic episodes in the deserts of southwestern North America. *Nature*, **320**, 441–444.

Thompson RS, Van Devender TR, Martin PS, Foppe T, Long A (1980) Shasta ground sloth (*Nothrotheriops shastense* Hoffstetter)

at Shelter Cave, New Mexico: Environment, diet, and extinction. *Quaternary Research*, **14**, 360–376.

Van Devender TR, Spaulding WG (1979) Development of vegetation and climate in the Southwestern United States. *Science*, **204**, 701–710.

Vasan S, Zhang X, Zhang X *et al.* (1996) An agent cleaving glucose-derived protein crosslinks *in vitro* and *in vivo*. *Nature*, **382**, 275–278.

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