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FEATURES OF DEEP CAVE SEDIMENTS: THEIR INFLUENCE ON FOSSIL PRESERVATION

T. Torres*, J. E. Ortiz* y R. Cobo**

ABSTRACT

We analyse how physical and chemical deep-cave sediment features preserve the morphological and geochemical characteristics of paleontological materials. Detrital sediment chemistry and clast size are fundamental because they provide a soft, impervious and plastic environment in which fossil remains are transported with minimal erosion. Sediment mineralogy provides a carbonate- and phosphate-buffered environment in which molecules of biological origin hydrolyze slower than in open-air environments or even at cave entrance sites. Because permafrost did not develop in the Iberian Peninsula (at least at the altitudes of inhabited caves), sediment desiccation never took place. In addition, sediment —pores were not aerated, which protected fossil remains from air (oxygen)-linked weathering. The annual-temperature variation inside sediment was negligible, which contributed to amino acid racemization dating. Collagen amino acid and amino acid racemization analysis of cave bear and man samples from cave sediments dated from different Oxygen Isotope Stages (4th: Sidrón, Amutxate, Troskaeta, El Toll, Coro Tracito, Ekain, Lezetxiki, La Pasada, Eirós; 5th: Reguerillo and Arrikrutz; 6th-7th: Sima de los Huesos) demonstrate that important amounts of almost intact collagen still remain in teeth dentine. Fossil DNA search seems to be very promising.

Key words: *Taphonomy, karst sedimentology, inorganic geochemistry, organic geochemistry, temperature variation, dentine, collagen, aspartic acid, racemization.*

RESUMEN

En este trabajo se analiza el papel que juegan las características físicas y químicas de los sedimentos de galerías profundas de cuevas en la preservación de los caracteres morfológicos y paleobiomoleculares del material paleontológico incluido en dichos sedimentos. Los aspectos geoquímicos y de tamaño de grano del sedimento son críticos: las características generan un medio blando, plástico e impermeable que permite el transporte —mecánico sin grave deterioro del material (en coladas de barro); las características químicas mineralogía del sedimento— proporcionan un ambiente con tampón fosfatado-carbonatado en el cual las biomoléculas hidrolizarán a un ritmo mucho más pausado que como lo harían al aire libre o en la entrada de las cavidades. Dado que el permafrost no fue un fenómeno extendido en la Península Ibérica durante al Cuaternario, al menos en las cotas a las que se situaban las cuevas habitadas por osos u hombres, nunca se produjo la desecación del sedimento y los poros no se llenaron de aire, protegiéndose los restos fósiles de la meteorización. Se ha registrado el cambio anual de temperatura dentro del sedimento y éste es prácticamente inapreciable, lo que añade nuevo interés a la fiabilidad del método de datación por racemización de aminoácidos. El análisis del colágeno remanente en la dentina de dientes de osos y hombres y de la racemización del ácido aspártico como herramienta geocronológica en material proveniente de sedimentos datados en diferentes Estadios Isotópicos del Oxígeno (4.º: Sidrón, Amutxate, Troskaeta, El Toll, Coro Tracito, Ekain, Lezetxiki, La Pasada, Eirós; 5.º Reguerillo y Arrikrutz; 6.º-7.º Sima de los Huesos) demuestra que cantidades importantes, o notables, de colágeno intacto todavía permanecen en la dentina, lo que hace que la búsqueda de ADN fósil parezca muy prometedora.

Palabras clave: *Tafonomía, sedimentología kárstica, geoquímica inorgánica, geoquímica orgánica, variación de temperatura, dentina, colágeno, ácido aspártico, racemización.*

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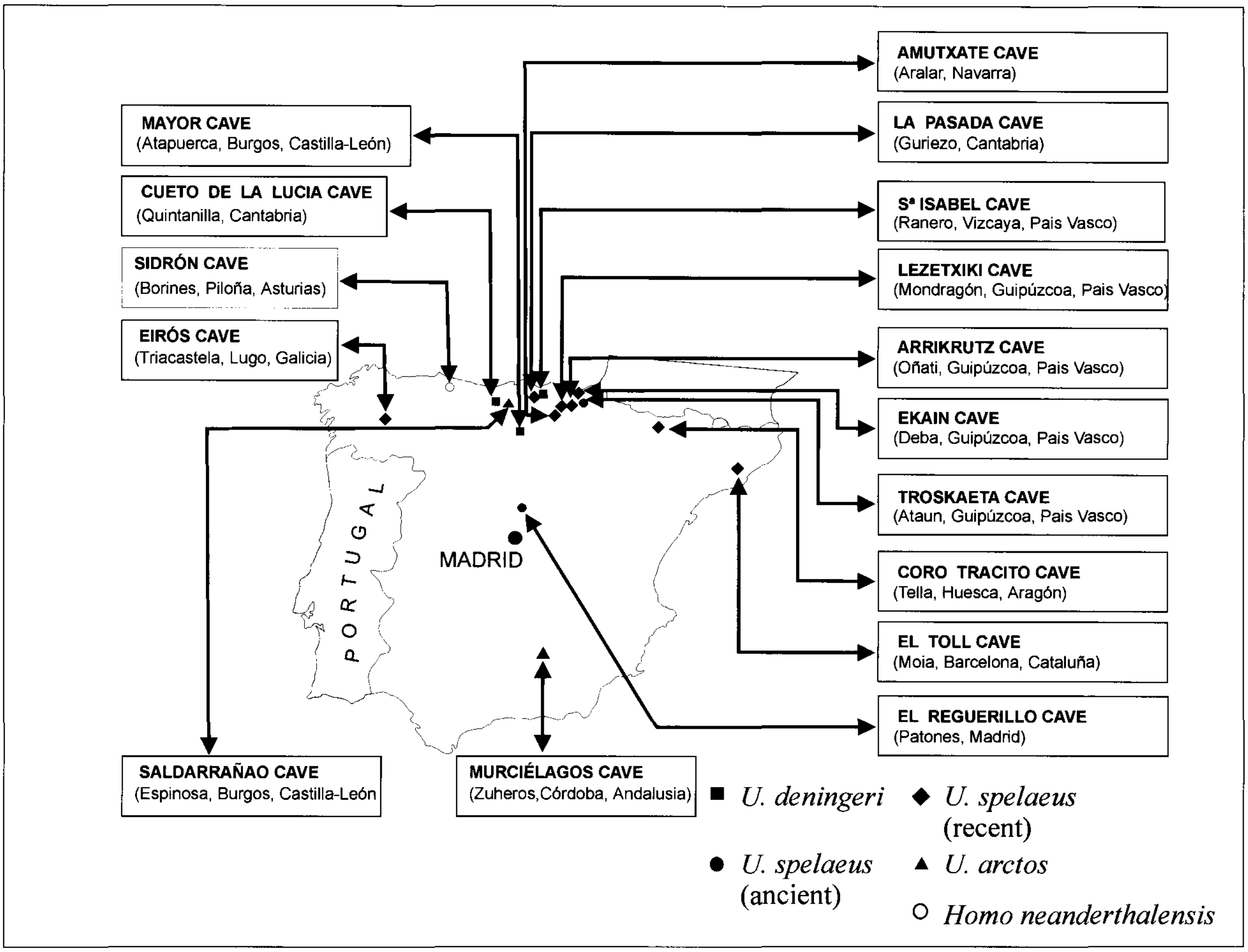

Fig. 1.—Geographical situation of the localities studied, including bear and human species.

Introduction

Strikingly, bony material found in deep-cave environments sometimes looks so fresh that it is a very hard work to convince the «paleontological media» that the findings are as old as they really are. This excellent conservation includes not only the outside appearance of bones and teeth but also their organic components such as collagen and other biomolecules. This paper deals with the influence of sediment characteristics on both physical and biological preservation of fossil vertebrate remains.

Area of study

In this paper we analyse biomarker preservation (*n*-alkanes and amino acids) from paleontological remains and sediment in some Spanish caves (fig. 1), mostly in the Northern half of the Iberian Peninsula. We analysed cave bear remains from various caves and ages. We also included some results of the biomarker analysis of *Homo neanderthalensis*, found in El Sidrón Cave (Asturias). We measured the temperature variation in two caves (El Reguerillo and Amutxate caves), and sediment granulometry and mineralogy in Amutxate cave.

Methodology

pH

The pH values measured in the Amutxate cave (Aralar, Navarra) sediments were measured after the sample moisturing (10 g of sample with 2.5 cc of water added) and after continuous magnetic stirring over-night.

Granulometry

Samples were dried and sieved at 2 mm and, then organic matter was removed with H_2O_2 and centrifuged. The remaining material was dispersed in destilled water and centrifuged. This process was repeated until the total defloculation of the sediment. Later on the sediment is sieved. The lutite fraction which was not retained, was analysed in a particle analyser (Coulter LS200).

Thermometry

The temperature variation inside the sediment was measured burying a Hobo H8 temperature recorder in 25 cm-deep holes in two caves: Reguerillo (Patones, Madrid) and Amutxate (Aralar, Navarra).

*Biomarker analysis (*n*-alkanes)*

Samples were first grinded and biomarkers extracted following the LEB's protocol which, in short, consists of (Lucini *et al.*, 2000):

The samples were introduced in an extraction cartouche made of quartz fibre previously ashed at 750 °C.

For the 24 h soxhlet extraction dicloromethane and methanol 2:1 (suprasolv Merck) were employed. After the extraction the isolated bitumen was concentrated using a rota-vapor device. Different bitumen fractions were extracted through liquid chromatography in a silica-alumina glass column using three solvants of different polarities: hexane, dicloromethane/hexane 80% and methanol.

After a new rota-vapor drying of the obtained fractions, 1 ml of DCM was added being placed in an autosampler vial, being automatically injected in a HP 6890 whit selective mass detector HP 5973 and column HP-5MS. The biomarkers were identified with a Wiley Library.

Biomarker analysis (amino acid racemization)

For amino acid racemization dating purposes dentine samples were recovered. The first step of our sample preparation method (Torres *et al.*, 2000), was focused on the collagen purification through a dialysis (at 3500D) process, which eliminated salts, free amino acids and low molecular weight molecules from CP and NCP diagenesis, before the sample preparation protocol which is described in Goodfriend (1991) and Goodfriend and Meyer (1991) and involves:

1. Hydrolysis which was performed under N_2 atmosphere in a hydrochloric acid for 20 h at 100 °C; later the samples were then desalted in HF and the resultant supernatant frozen and dried under vacuum.
2. Derivatization; amino acids were derivatized in a two step process, involving first esterification with thionyl chloride in isopropanol and acylation.

1-4 aliquots μl were injected into a Hewlett-Packard 5890 gas chromatograph with a Chirasil-L-Val fused silica column (0.39 mm x 0.25 μm x 25 m) from Chrompack.

Results and discussion

Two main facies can be distinguished in cave infills: entrance facies and deep gallery facies (Jennings, 1985).

As entrance facies are controlled by external agents, entrances have a wide spectrum of palaeontological and archaeological deposits. In the cave entrance, sometimes a wide hall, there is a continuous arrival of allochthonous materials from gravitational processes (fall, roll, creep, flow and slide). These materials are rapidly mixed with biogenic materials from predators, scavengers or cave occupants. Sometimes the human occupation of cave entrances produced a debris cone dipping outwards or even inwards. At any rate, during the Pleistocene periods of periglacial climate very important frost wedging created thick regoliths (cryoclast accumulations) which later sunk into the cave entrance. This happened at the Gran Dolina in Atapuerca (Burgos). Cave entrance deposits are usually very porous, lying on local impervious bedrock basement. This caused capillary fringe depth oscillation, reflected in the weathering of the stacked sediment beds which change to reddish shades.

In many cases, deep-cave galleries had a previous stage as a branch of a karstic network under phreatic conditions (Jennings, 1985). Then boundary conditions changed, i.e. the base level falls de-activated the uppermost phreatic galleries, which then functioned as ponors through allogenic rivers torrented into the now open cave galleries. The Reguerillo cave in Madrid (Torres, 1974; Torres *et al.*, 1993) is a typical case of an allogenic river (Lozoya river) with headwaters on impervious granitic and metamorphic rocks that entered in the cave transporting gravel and sand-sized sediments. Another example is the Eirós cave (Triacastela, Lugo) where aepigean running waters torrented inside the cave after the spring thaw and were responsible for both the gravel deposits and the rolling and transportation of paleontological remains deposited as bioclastic channel bars (Torres *et al.*, 1991a). In the Conturines cave (Italy) an autogenic stream, formed by thaw, torrented into the cave transporting a «dolomitic sandy gravel» from the weathered karst rock (dolostone) which lacked lutite-size matrix (Rabeder, 1992).

The most frequent sediment accumulation in deep-cave galleries, consists of lutitic sediments massively, crudely or little embedded with scattered sand, gravel and boulder-sized intraclasts as a result of fall processes from ceilings and walls. The origin of cave lutites may well be polygenic: there are definitely varying contributions from different environments.

Fine-grained sediments are reddish-brown (10 R 4/6) or brown (5 YR 4/4), but fully red shades are almost absent. We found red lutites interbedded in Amutxate cave (Aralar, Navarra). In fact, Torres (1974) describes «unborn» pendants, which were developed on bedding planes, where anastomosed carvings created by dissolution, retained the cave lutites generated after the terrigenous-rich dolostone dissolution.

According to Bretz (1942), cave lutites could have been introduced into the caves from surface soils («terra fusca» or «terra rossa») during resisthasic periods when soil destruction and fluvial incision were enhanced and further vadose modification of the caves occurred. This hypothesis can help to explain the presence of some cave lutite mounds at the foot of a vertical joint, cutting the main phreatic gallery: in this case, there is a real residual deposit. In conclusion, in deep-cave galleries thick cave lutites consisting of detrital clay minerals and silt-sized quartz are the most common infill type.

Deep-cave sediments are usually more homogenous in composition, but due to the complexity of karst history they can also produce complex stratigraphies. Their final composition is an integration process of two sources of autogenic sediments: inputs from karst rock weathering and biogenic inputs (usually from cave-inhabitant or cave-colonist organisms). Allogenic inputs from sinking rivers may also be common.

Karst rock dissolution produces a variety of soluble compounds which after their transportation along rock joints, usually precipitate as calcite and/or aragonite and, rarely, gypsum. They build speleothems and, in some cases, calcite-sinter, cementing formerly accumulated clastic sediments. In some cases «juvenile stalactites» (macarroni or soda straw stalactites), which grow under favourable conditions, later fall and become part of the clastic fraction of the deep-cave sediment, allow dating (^{14}C or U/Th). In any case, speleothems as flowstone or sinter-makers are a real nightmare for archaeologists and palaeontologists because they make the extraction of entombed fossil remains very difficult, obliging them to resort to chemicals (acetic acid) for fossil extraction.

Due to the polygenic origin of sediments, sediment sorting is extremely poor. It usually consists of lutites mixed with clasts, with a maximum diameter of many meters, and bioclasts, which can be nearly 1m long.

The lutite fraction predominantly consists of silt-sized materials (fig. 2). Clay-sized particles never exceed 18% of sediment weight. Similarly, fine sand-sized clasts never exceed 20% of sediment weight. The dominant sediment particle size explains, whether the materials accumulated mainly in a low-energy environment such as an underground run-off. In the Amutxate cave granulometrical analysis, three clusters were distinguished. The dominant grain size has an average value of 40 µ; another clast size distribution has around 20 µ average size; and a third one, around 8 µ. The

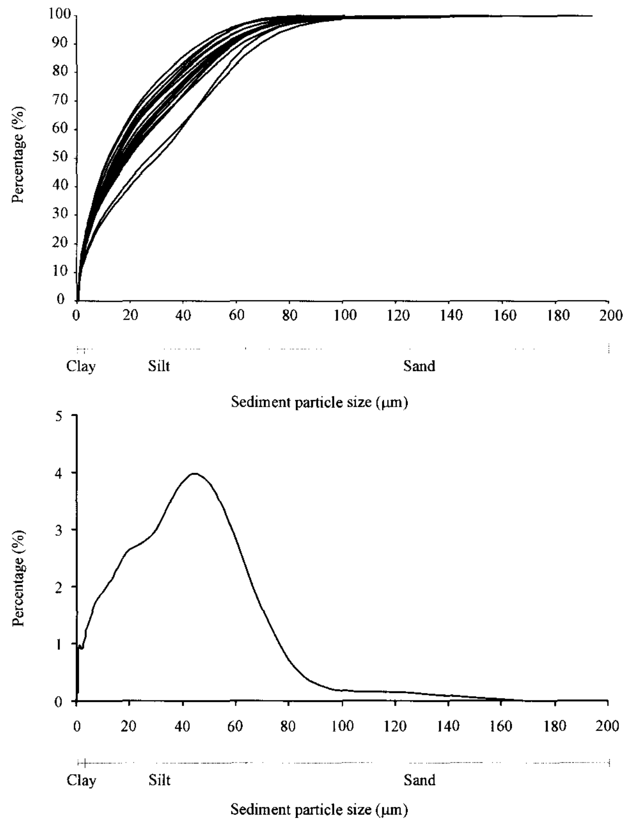

Fig. 2.—Granulometrical analysis of < 1mm sediment fraction from Amutxate cave. **A)** granulometrical (accumulation percentage) curve of all Amutxate cave sediment samples. **B)** A single non-accumulative granulometrical curve showing the typical «polymodal» aspect common in all Amutxate cave samples.

dominant particle size represents the main down-slope flow sediment contribution whilst the other two represent finer lateral inputs after infill surface washing from extremely low-energy laminar runoffs.

The breakdown of cave roof and walls was responsible for piles of angular blocks that were not embedded. According to Jennings (1985), these materials are cone-shaped when located below a roof dome or collapse-doline, extending as block streams towards the innermost parts of the caves. In our experience, there is a continuous «clast rain» in all cave sub-environments, which may be due to weathering and tension crack development caused by stress in Trompeter's decompressed zone, the result of water support loss after base flow descent. Joints and faults, which are ever-present in fragile limestone, favour gravitational clast inputs. In all our palaeontological excavations of the past 30 years, we have observed that boulder and gravel-sized clasts associated with bone-bearing beds are very poorly rounded, with blunted rather than angular-shaped edges. This cannot be explained as a transportation-linked process, which in many cases can be estimated as less than ten meters in length. Fallen blocks are blunted in place due to subsoil dissolution by non-bicarbonate-saturated waters from superficial runoff or dripping. This dissolution contributes to high-calcium carbonate contents in the lutitic matrix (epimatrix) which is very effective in buffered sedimentary environments maintenance, further favouring down-slope mass movement. The presence of thin calcium-carbonate films coating the «upstream face» of buried fossil remains is evidence of calcium bicarbonate saturated underground water circulation.

Mass movements, whether sliding or flowing in nature, are very conservative transportation processes, that can move fossil remains without marked disturbances if they are anatomically connected and there is no-mechanical erosion, although sometimes they get scratched. We explain bone destruction as a chemical-weathering process due to capillary action-water circulation through bone micro-fissures. It is extremely significant that in the Amutxate cave where some cave bear skulls were partially sediment-covered, their aerated parts were corroded, despite the appearance of dissolution holes. Air-exposed skull parts look dusty due to calcium-carbonate accumulation through capillary action. Two ulnae seemed literally thrust into the sediment with their driven parts well preserved and their free ends, the styloid apophyses, looking like «half burnt candles». Due to the sediment's mass movement almost all the bones go through this process (open-air weathering) during a highly variable time span, and may break or even be crushed by fallen rock from the roof.

Taphonomical evolution of fossil accumulations in deep-cave galleries is a very complex phenomenon. We can divide all fossil accumulation into a multi-step process that takes place in four different areas:

1. Biogenic sediment factory. Area of human habitation and animal dens.
2. Transfer zone: where the accumulation of fossils is stable for a short time-period.
3. Accumulation zone: where the archaeological/palaeontological site is formed.
4. Destruction zone. Sometimes the accumulation zone suffers weathering-erosion by air exposure caused by sediment drying or after erosion. A typical result is an accumulation of almost nothing but teeth.

Table I.—**pH and % $CaCO_3$ values in samples from Amutxate cave**

Sample	pH	% $CaCO_3$ (<100μ)
AX-A1-10	8.0	30.09
AX-A1-40	7.8	18.31
AX-A1-60	7.4	17.02
AX-A1-80	7.2	18.00

Inorganic geochemistry and mineral composition

The character of the pH of cave sediments is crucial to the preservation of the mineral and organic components of fossils. The pH values measured in the Amutxate cave (Aralar, Navarra) sediments range between 7.2 and 8.0. The highest pH values (8.0-7.8) are reached in the shallower samples analysed (10 cm and 40 cm deep) both from cave bear bone accumulation, while the other sample (60 and 80 cm deep) was taken from paleontological-sterile sediments.

The calcium-carbonate content was measured in the same samples (see table I). High calcium-carbonate contents still remain in the cave lutites as fine-very fine sand-sized and silt-sized sediments. The high carbonate-content explains the pH values measured and suggests that the sediment is a chemically buffered environment allowing good bone and teeth preservation. In the case of Amutxate cave sediments the sudden decrease in $CaCO_3$ content from the shallower sample to the deepest ones is noteworthy.

Calcium carbonate, calcium phosphate, quartz grains and detrital clays are dominant. There are also some minerals such as pyrite (sometimes botryodal), ilmenite, magnetite, hematites, etc. Among detrital clays, illite, montmorillonite, chlorite and caolinite appear (Torres, 1974). Microfossils and macrofossils inherited from the karst rock can be very abundant.

Thermometry

Hoyos *et al.* (1998) found that annual air temperature variation in the Candamo cave in the northern part of Spain was almost negligible in deep-cave galleries or halls (less than 1 °C year^{-1}). To check the possible annual, or even seasonal, temperature change inside the sediment affecting the preservation of fossil remains we recorded the sediment temperature variation in the Reguerillo cave (Patones, Madrid) and in Amutxate cave (Aralar, Navarra) (fig. 3). Although the open-air inter-seasonal

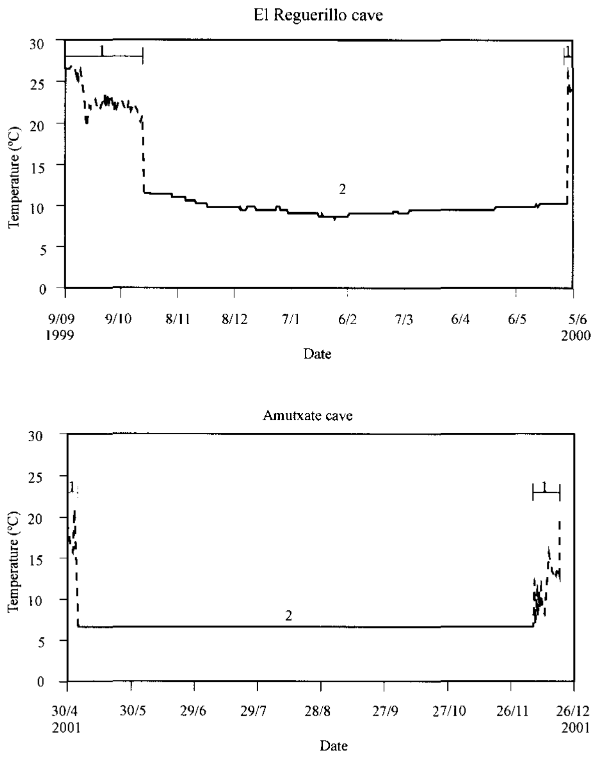

Fig. 3.—Graphs of temperature record inside the sediment at 30 cm deep (2) of two localities: El Reguerillo cave, near Madrid with an annual average temperature of 12.5 °C, and Amutxate cave in Navarra, where the open air annual average temperature is between 7-8 °C. Due to the loggers needed to be sealed in the lab, a wide record of open air temperature was also registered (1) before and after the logger burial into the sediment.

temperature variation is higher than 10 °C, in both cases almost no temperature variation was recorded. Moreover, in Amutxate cave an excavation during the temperature-recording period involved 10 persons plus five powerful floodligths in the hall where cave bear remains were unearthed.

Organic geochemistry

It was to be expected that a large number of biomarkers would be found in the lutites where mammal remains are scattered as well as within the mammal remains themselves. In both cases the biomarker-preservation potential lies in the physical and chemical characteristics of the sedimentary environment. A combination of protection from air exposure and basic buffered environment seems to be crucial. Both are common in deep-cave galleries.

The Amutxate cave infill consists predominantly of lutite-sized sediments. These lutitic sediments had a double provenance. First, they reached their deposition site as mudflows —some recent mudflow tongues are still visible— from a thick marl bed overlying the Cretaceous limestone formation where the cave developed. Second, some coarser inorganic components also appeared: sand-to-pebble size-clasts, sub-rounded to sub-angular in shape due to «on-ground» chemical weathering (karst rock weathering), and angular pebbles-to-boulder size-clasts from roof and wall falls.

Three lutite samples and one gravel one were analysed to check biomarker preservation. The results from Amutxate cave sediments (see fig. 4), reveal that the Amutxate-Z3 sample, formed by grinded gravel-sized clasts, gives the biomarker contents of Cretaceous karst rock (black micritic limestones). There is a significant balance among the abundance of *n*-alkanes with even and odd carbon atoms and also a «hump» or unresolved complex mixture (UCM) extending over much of the range occupied by *n*-alkanes. This can be interpreted as a mature, old, oil-source rock where *n*-alkanes have cracked over time (Killops and Al-Juboori, 1990).

The chromatograms from three sediment samples, taken at different depths (7, 17 and 27 cm) in an exploration trench dug at the A1 excavation square, reveal a different biomarker pattern. Short chain *n*-alkanes are lacking or appear in very low concentrations whereas long-chain *n*-alkanes with odd carbon atoms (*n*-c27, *n*-c29 and *n*-c31) appear in remarkably high abundance.

The chemical weathering of the karst rock may be responsible for the total loss of pre-existing old biomarkers (of the lower Cretaceous age) cracked and finally destroyed during this process, which transformed hard limestone into soft and fine-grained lutites.

The above data, establish certain preliminary features of the physical and chemical aspects of deep-cave galleries:

— In taphonomical terms, specific mammal bone accumulations are frequent in deep-cave galleries.

— Contamination from open-air provenance seems to be uncommon.

— Bioclasts usually appear in a lutitic matrix derived from karst rocks weathering. Mineral neoformation, espeleothems excluded, is not common with the sole exception of a cryptochrystalline apatite variety (collophane) which usually forms noduli as in the Gran Dolina at Atapuerca, Burgos (Hoyos pers. com., 1976). These noduli are derived from former mineral tissue-fragments, hydrolysis and further deposition.

— Although sediment can inherit biomarkers from ancient karst rocks, the most important bio-

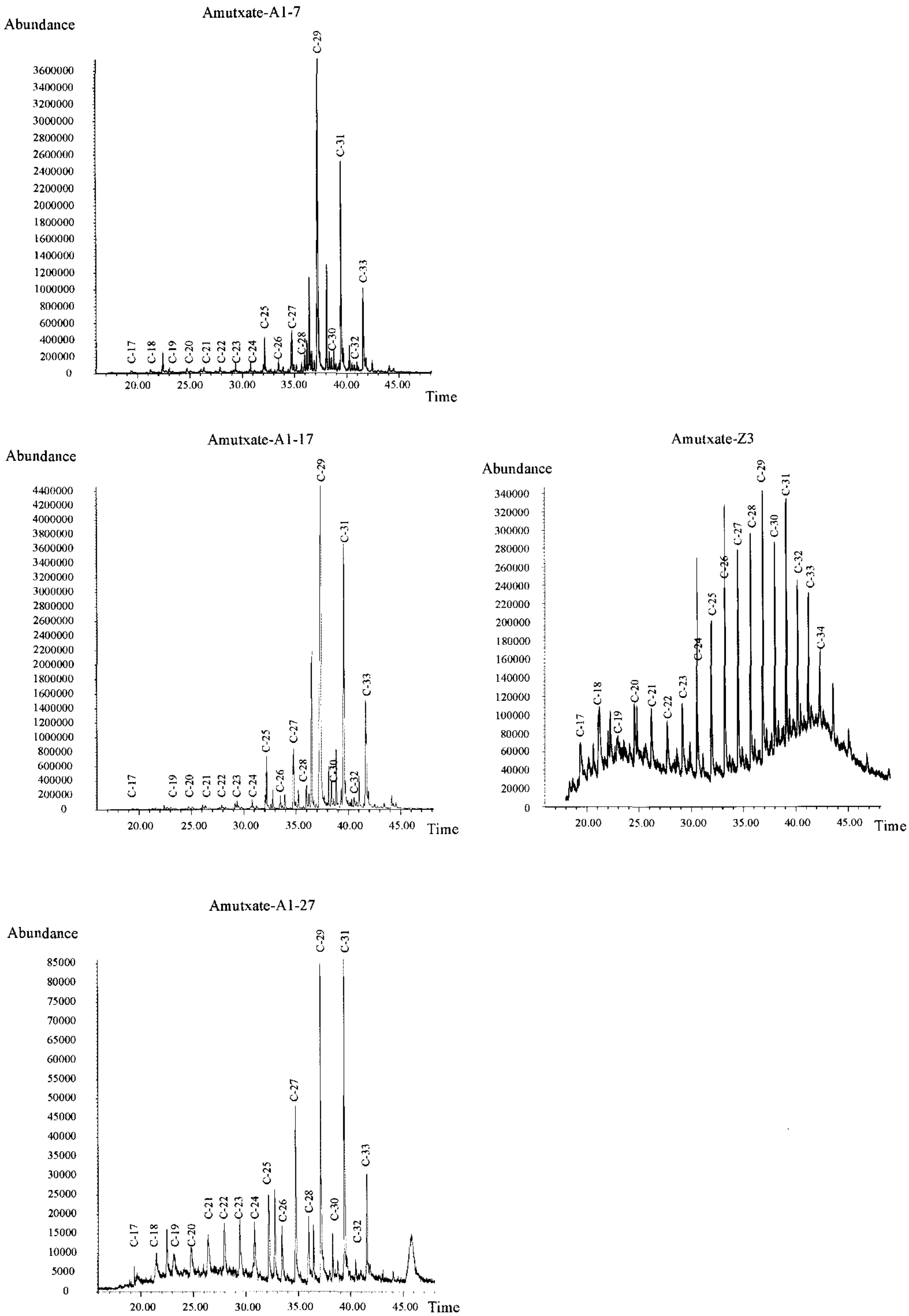

Fig. 4.—Some examples of biomarker preservation (chromatograms with the *n*-alkane distribution) in Amutxate cave sediment. Samples Amutxate-A1-7, 17 and 27 were picked at different depths (7, 17 and 27 cm) in a trench digged at the A1 excavation square. Amutxate-Z3 consisted of grinded gravel-size clasts from the Lower Cretaceous karst rock which could be considerated as a good oil source rock, recovered at the Z3 excavation square because of the «hump» or unresolved complex mixture (UCM) and the balance among the abundances of *n*-alkanes with even and odd carbon atoms.

marker source is vertebrate soft and hard tissue decay, or any other foreign biomarkers afforded by cave inhabitants.

— Lutitic matrix can be viewed as highly porous and not very permeable environment, ensuring a constant moisture presence around vertebrate remains and insulating them from atmospheric oxygen.

— Chemically, the basic features of lutites are permanently ensured by the buffer role played by the still-remaining calcium carbonate. The presence of collophane noduli can be considered an indicator of an, at least temporarily, phosphate-buffered environment.

— Temperature variations inside the fossil bearing beds are negligible.

Some previous consequences of preservation vertebrate remains are:

— Some paleontologists and paleoanthropologists seem really shocked when they observe the incredibly good preservation of vertebrate remains found in deep-cave gallery infills. This usually occurs when lutites are not carbonate-cemented, such as the Middle Pleistocene human and bear remains from the Sima de los Huesos (Atapuerca, Burgos) or the oldest hominid remains from Dmanisi (Georgia) (Gabunia *et al.*, 2000).

— This exquisite preservation can be explained by the protective influence of the above mentioned favourable physical and chemical environmental characteristics of deep-cave galleries. Outstanding examples are the new skull discovered at Dmanisi in Georgia (Vekua and Lordkinipanidze, 2002) and the newborn bear skeletons of Troskaeta cave in northern Spain (Torres *et al.*, 1991b).

— In addition, the lutitic matrix acts as an extremely good preserving environment when mass flow movements, very common in caves, occur, as it protects bone and teeth from erosion or breakage. In some cases, as in Troskaeta (Torres *et al.*, 1991b) articulated parts of skeletons were safely transported as a whole.

— Biomarkers in sediment (lutites) are also preserved for a long time.

— What happens to vertebrate-linked biomarkers, such as collagen, DNA, osteocalcin as well as a number of peptides of different chain lengths and even free amino acids?

The biomarkers appearing in the lutitic cave infill samples can be interpreted as the result of the decay of recent, Pleistocene organic matter (dominance of long *n*-alkane molecules with odd numbers of carbon atoms). The classic interpretations is that the presence of these biomarkers is explained by plant-tissue decay. However, in deep-cave galleries it seems unlikely that plant litter is dominant. A more reliable alternative interpretation is that the biomarkers come from bear-tissue decay. The small amounts of *n*-alkanes with even C-atom numbers can be interpreted as a reflection of early recent organic matter diagenesis or a relict input from Mesozoic black limestone. Samples Amutxate-7 and Amutxate-17 came from the fertile palaeontological layer, while sample Amutxate-A1-27 was recovered in an almost sterile layer whose deposition was coeval with the beginning of the cave occupation by the cave bears and, consequently, biomarkers (*n*-alkanes) appear at lower concentrations ($\approx$ 20%). Nevertheless, deep-cave galleries, with fine-grained sediment infills, are very favourable environments for plant biomarker preservation. Old biomarkers from karst rock were almost totally destroyed by the weathering (air-rock or water-rock) interface.

One of our bigger challenges was to ascertain the survival opportunities of organic molecules in vertebrate bony remains, including human beings, which are frequently scattered in the lutitic infills of deep-cave galleries. Due to their «evidently more favourable preservation potential», we chose to focus our research on biomolecule preservation in cave bears' dentine, with some short incursions into the «cave bear bony kingdom and old humans' dentine realm».

In teeth dentine, type-I collagen amounts to 90% of the organic matrix. Teeth dentine also has non-collagenous proteins (NCP) such as glycoproteins, γ-carboxy-glutamic-acid (osteocalcin), phosphoproteins and proteoglycans (Linde *et al.*, 1980). Francillon-Viellot *et al.* (1990) cited type-I collagen, type-V collagen and elastoidin as collagenous components, as well as a wide variety of non-collagenous components: proteoglycans, sialoproteins, glycoproteins, phosphoproteins, osteocalcin, osteonectin, serum proteins and lipids.

The Biomolecular Stratigraphy Laboratory of the Madrid School of Mines uses dentine almost exclusively for amino acid racemization dating purposes. The use of bones is rejected because they are more prone to early diagenesis which is reflected in the appearance of spurious racemization ratios as noted by Masters (1987).

It is not our purpose here to describe amino acid racemization as a proxy-dating tool (Torres *et al.*, 2002), but to discuss the preservation over time in deep-cave gallery environments of the dentine organic matrix as a vertebrate fossil DNA preservation indicator.

Outstanding amounts of amino acids were found in bear dentine samples with ages ranging from ten

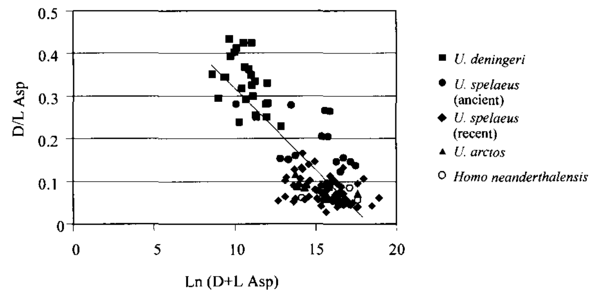

Fig. 5.—Plot of the aspartic acid racemization ratio against D+L aspartic acid chromatogram peak areas (corrected for sample weight and injected sample volume) of *U. deningeri*, *U. spelaeus* (from ancient localities and recent localities) and *U. arctos* dentine collagen samples from different localities of the Iberian Peninsula. Values from *Homo neanderthalensis* samples from El Sidrón cave were added (modified from Torres *et al.*, 2002). See fig. 1 for cave locations.

thousand to more than two hundred thousand years (Torres *et al.*, 1999). A net correlation between D/L Asp racemization ratios and total Asp (fig. 5) was also found (Torres *et al.*, 2002). Because collagen amounts to at least 90% of organic matrix in dentine, our interpretation was that amino acids from dialyzed samples have a collagenous source in spite of the samples' steady age-linked amino acid loss. To reduce uncertainty, we worked to discard any possibility that amino acids had a non-collagenous source. When Ajie and Kaplan (1991) demonstrated that osteocalcin was more suitable than collagen for isotopic analysis, they used the lack of hydroxyproline, which was very abundant in Collagen I composition (Francillon *et al.*, 1990), as a test to check the absence of collagen or collagen- peptide contamination.

Thus, the presence of a conspicuous L-Hyp peak in all dentine samples analysed certifies that important amounts of collagen (molecules bigger than 3500 Da) are preserved for an unexpectedly long time in deep-cave gallery environments. As osteocalcin molecules are more stable than collagen ones, they too survive. Possibly DNA may too.

The progressive decay of organic-mineral dentine components is not a linear process. In the most recent cave bear (4th OIS) canine dentine there is a progressive loss of total aspartic acid (collagen-linked) from the «outermost» parts of the root (cement wall and pulp channel) toward the central part of the root body. And this differential geochemical behaviour also affects the D/L Asp ratios. This was interpreted as a progressive cracking of collagen molecular bonds, which allow higher racemization rates, strongly constrained by the tighted triple helix structure of the intact collagen. In fact, this decay is an expression of the unavoidable weathering processes, which even take place in favourable environment such as deep galleries. In not so recent cave bear teeth (5th OIS), important collagen structure delay occurred and a more regular distribution of intact collagen-linked aspartic acid (retained in the sample after 3500 Da dialysis) and aspartic acid racemization ratios was found. Although some differences still appear-near the cement wall of the root the lowest total-Asp concentrations and highest D/L Asp ratios were measured. In very old bear remains (6th-7th OIS) there is an equivalence of both D/L Asp ratio values and total Asp contents (in the hydrolisate after the 3500 Da dialysis had removed all free amino acids, dipetides and short-chain polypeptides).

The above findings explain the high D/L ratio standard deviation values calculated from sample sets from the same bed. This makes it necessary to analyse a high number of samples. In addition, more samples are better than single racemization values for age calculations.

Conclusions

Deep-cave galleries are unusually, a highly favourable environment whose physical and chemical characteristics allow surprisingly good physical preservation of palaeontological and paleoanthropological remains. Stable conditions of temperature, sediment moisture, sediment pH and chemical composition (carbonate and phosphate-buffered) make the preservation of biomarkers in dentine and, even, in bones very possible. It is an encouraging scenario for ancient DNA search. Despite these outstanding preservation characteristics, continuous organic-molecule hydrolysis occurs. This process ends after the preservation characteristics′ total loss, but does not occur in a linear fashion. A strict sampling protocol, including sampling the central part of either the internal or the external root wall, is required. This makes the use of linear or non-linear models for radioactive s.l. dating a source of further uncertainty.

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