



MAMMAL REMAINS

Dating of mammal remains from Spanish caves and open air sites. Teeth dentine.

The amino acid racemization method has been proved to be a useful tool for dating teeth of mammal remains. The use of bones is rejected because they are more prone diagenetic interferences (Masters, 1986).



Cave bear right mandible (male adult) from Cueva del Reguerillo
(Madrid Central Spain) dated at ca. 150 ky

Pleistocene brown bear group species (*Ursus etruscus* G. Cuvier, *Ursus prearctos* Boule and *Ursus arctos* Linn.) covered the whole of the Iberian Peninsula while cave bear species (*Ursus deningeri* Von Reich and *Ursus spelaeus*) were restricted to their northern part, where Euro-Siberian climatic conditions prevailed over Mediterranean ones. The main questions to solve were: when they colonized the Iberian Peninsula and what they represented in the Pleistocene evolutionary tree. The analysis of the racemization ratio of the aspartic acid obtained from the collagen of bear teeth, mostly canines, dentine has been proved to be a useful tool for species validation and dating (Fig.1). According to our recently obtained data, *Ursus deningeri* was a genuine Middle Pleistocene representative which cannot be identified as a synonymous or sister species of the common cave bear (*Ursus spelaeus*) which colonized the Iberian Peninsula during two different time periods: the uppermost Middle Pleistocene and the Tardiglacial.

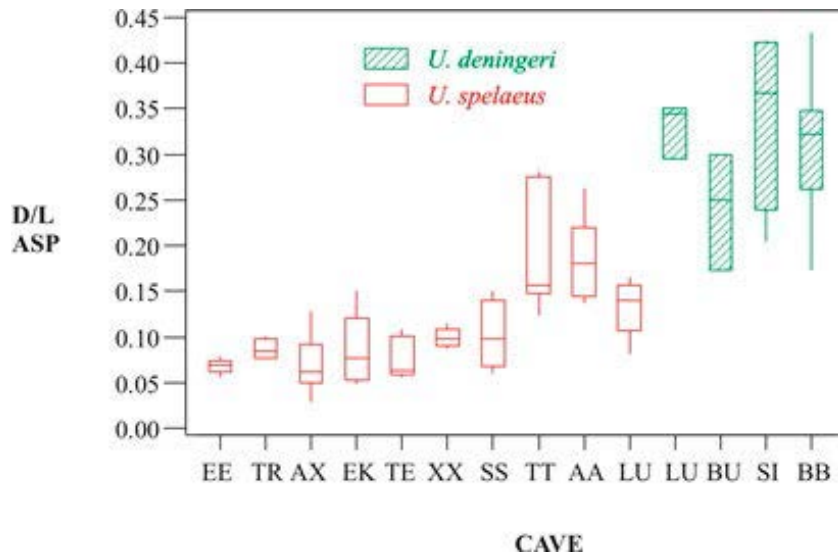


Figure 1. Aminostratigraphy of Iberian bear localities based on the box-and-whisker plot of aspartic acid racemization ratios of *U. deningeri* and *U. spelaeus* from the different sites studied.

Given that amino acid racemization is not a numerical dating method in itself, it requires calibration, mainly with radiometric dating methods. We have established the age calculation algorithm for aspartic acid D/L ratios in dentine collagen using samples taken from levels previously dated by ¹⁴C, U/Th, ESR (Torres et al., 2002) (Fig.2).

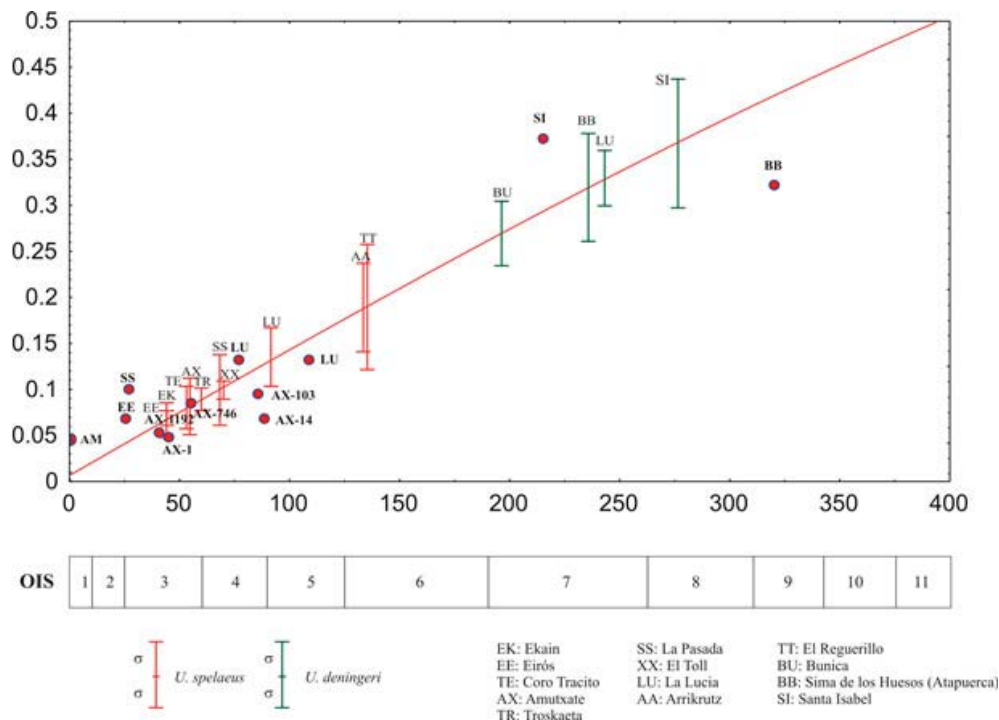


Figure 2. D+L aspartic acid peak areas histogram from bear teeth samples of different localities of Spain. *Ursus deningeri* samples are of Middle Pleistocene age. *Ursus spelaeus* samples are of Upper Pleistocene age. *Ursus arctos* samples are of Holocene age. The scale is semilogarithmic. Modified from Torres (1999)



Although monogenic samples are necessary to reduce taxonomically controlled variability in D/L ratios, it is verified that differences in racemization of mammal collagen samples are despicable. Therefore, this age calculation algorithm has been applied with good results to date Homo, Equus, Rhinocerus, Elephas, Cervus, etc samples from different localities (cf. Fortea et al., 2003; Navazo et al., 2005; Rosas et al., 2006; Díez et al., 2008; Torres et al., in press). When compared with intracrystalline proteins from molluscs, collagen from teeth dentine has a very low preservation potential being unprotected in a no-crystalline matrix. This makes very important paleoenvironmental conditions influence. In rock shelters and even in open-air sites diagenesis affects collagen integrity in a very early stage being usually hydrolyzed and resulting free amino acids leached. In deep cave environments a more lasting preservation was produced and “intact” collagen molecules have found in dentine dated ca. 350 ka (Fig.3)

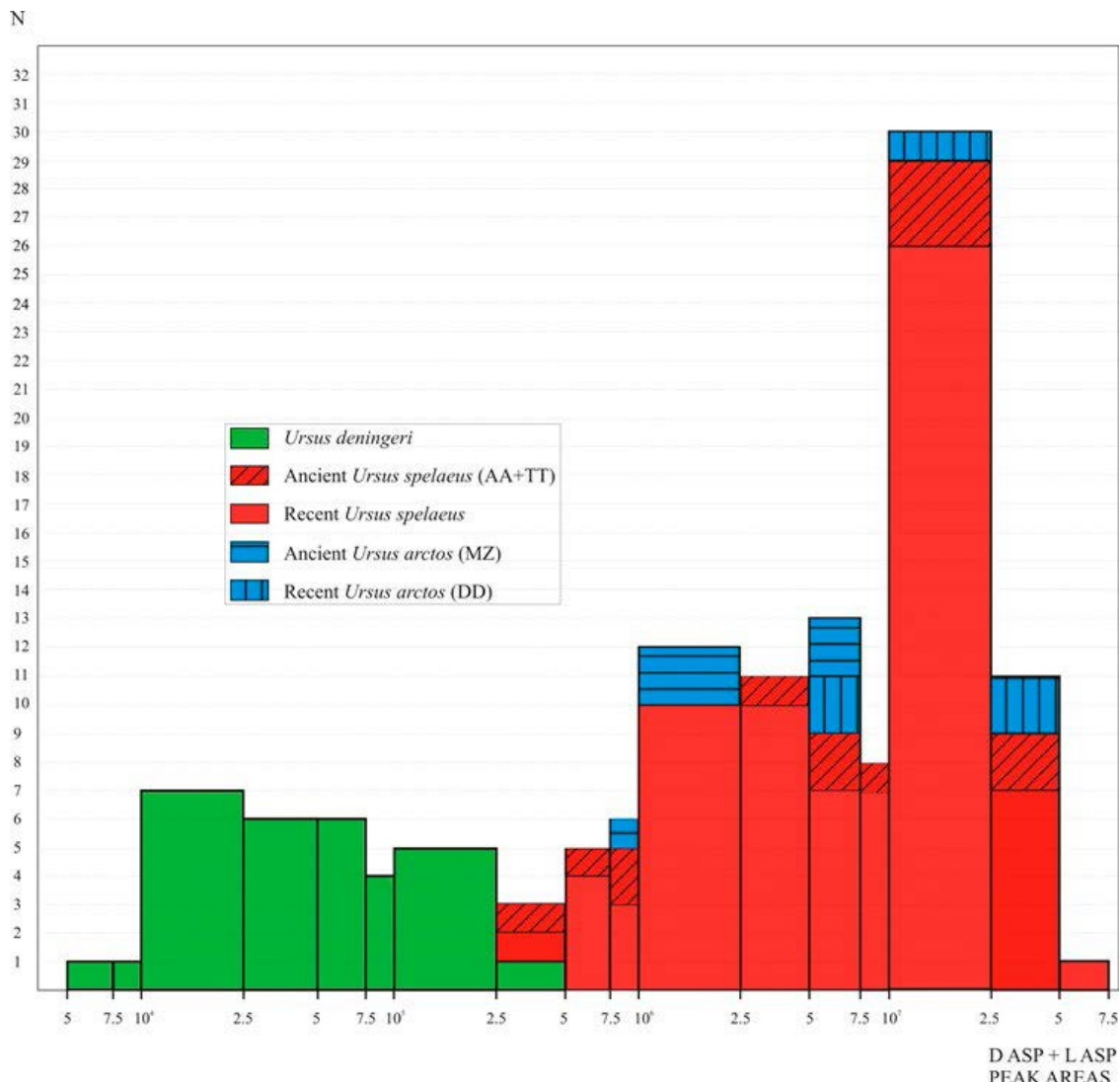
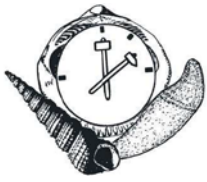


Figure 3. Dating of Spanish cave bear localities using the age calculation linear model for the D/L asp ratios. Modified from Torres et al. (2001, 2002). Circles represent the bear localities dated by different dating methods: ¹⁴C in bones (Eirós Cave, Galicia; Grandal d’Anglade and Vidal Romaní, 1997), Th/U in speleothems (La Lucia Cave, Cantabria; Torres et al. 2001b), electron spin resonance (ESR) and uranium series in bear teeth (Sima de los Huesos, Burgos; Bischoff et al., 1997) and unpublished ESR data obtained from bear teeth (Amutxate Cave, Navarra; Troskaeta and Santa Isabel Caves, Vizcaya; La Lucia and La Pasada Caves, Cantabria).



According to Julg et al. (1987) and Collins et al. (1999) the structural constraints are linked to the triple helix collagen structure, but based on our kinetics experiments, (Torres et al., 2001), the aspartic acid racemization is produced, even before the collagen denaturation, is reached. In nature, the racemization will be easier in products (short chain peptides or free amino acids) arising from the degradation of collagen by chemical and bacterial agents. Probably this can be explained in terms of geochemistry behaviour of the dentine in a very stable, in geochemical terms, environment where a continuous water inflow was produced into the root via two main ways: outer side (cement wall) and a central hollow (pulp cavity) at the same time that collagen molecules were hydrolyzed and free amino acids are leached along dentine growth layer and through dentinal channels. This process will be faster in more aggressive environments with changing thermal conditions, interstitial water salinity, total moisture, Eh and pH and can explain the lack of any collagen molecule in samples from lacustrine environments, such as in Venta Micena site, and rock shelters, such as Gran Dolina of Atapuerca. In our opinion the searching for well-preserved collagen molecules, is a decisive first step in the searching for fossil DNA.

References

Collins, M.J., Waite, E.R., van Duin, A.T. (1999). Predicting protein decomposition; the case of aspartic acid racemization kinetics. *Phil. Trans. Royal Society Series 354*, 51-64.

Díez, C., Alonso, R., Bengoechea, A., Colina, A., Jordá, J.F., Navazo, M., Ortiz, J.E., Pérez, S., Torres, T. (2008). El Paleolítico medio en el Valle del Arlanza (Burgos). Los sitios de la Ermita, Millán y La Mina. *Cuaternario y Geomorfología* 22(3-4), 135-157.

Fortea, J., Rasilla, M., Martínez, E., Sánchez-Moral, S., Cañaveras, J.C., Cuezva, S., Rosas, A., Soler, V., Castro, J., Torres, T., Ortiz, J.E., Julià, R., Badal, E., Altuna, J., Alonso, J. (2003). La Cueva de El Sidrón (Borines, Piloña, Asturias). Campañas Arqueológicas de 2000 a 2002. *Estudios Geológicos* 59(1-4), 159-179.

Julg, A., Lafont, R., Perinet, G. (1987). Mechanisms of collagen racemization in fossil bones: application to absolute dating. *Quaternary Science Reviews* 6, 25-28.

Masters, P.M., 1986. Amino acid racemisation dating. In Zimmerman, M.R., Angel, J.L. (Eds.), *Dating and Age Determination of Biological Materials*. Croom Helm, London, pp. 39-58

Navazo, M., Díez, C., Torres, T., Colina, A., Ortiz, J.E. (2005). La cueva de Prado Vargas. Un yacimiento del Paleolítico Medio en el sur de la Cordillera Cantábrica. Museo de Altamira. *Monografías* 20, 151-166

Rosas, A., Martínez-Maza, C., Bastir, M., García-Taberner, A., Lalueza-Fox, C., Huguet, R., Ortiz, J.E., Julià, R., Soler, V., Torres, T., Martínez, E., Cañaveras, J.C., Sánchez-Moral, S., Santamaría, D., Rasilla, M., Fortea, J. (2006). Paleobiology and comparative morphology of a late Neandertal sample from El Sidrón (Asturias, Spain). *Proceedings of the National Academy of Sciences* 103(51), 19266-19271.



Torres, T. (1999) . Amino Acid Racemization in Fossil Pleistocene Vertebrate and Invertebrate. Preservation of Proteins. In *Advances in Biochirality* (G. Palyi, C. Zucchi and L. Caglioti Eds): 247-256. Springer Verlag S.A.

Torres, T., Ortiz, J.E., García, M.J., Llamas, F.J., Canoira, L., García de la Morena. M.A., Julià, R. (2001). Geochemical Evolution of amino acids in Pleistocene bears. *Chirality* 13(8), 517-521.

Torres, T., Ortiz, J.E., Llamas, F.J., Canoira, L., Julià, R., García-Martínez, M.J. (2002). Bear Dentine Aspartic Acid Racemization Analysis, Proxy for Pleistocene Cave Infills Dating. *Archaeometry* 44 (3), 417-426.

Torres T., Ortiz, J.E., Cobo, R., de Hoz, P., García-Redondo, A., Grün, R. (2007). Hominid exploitation of the environment and cave bear populations. The case of *Ursus spelaeus* Rosenmüller-Heinroth in Amutxate cave (Aralar, Navarra-Spain). *Journal of Human Evolution*, 52, 1-15.

Torres, T., Ortiz, J.E., Grün, R.; Eggins, S.; Valladas, H., Mercier, N., Tisnèrat-Laborde, N., Julià, R., Soler, V., Martínez, E., Sánchez-Moral, S., Cañaveras, J. C., Lario, J., Lalueza-Fox, C., Badal, E., Rosas, A., Santamaría, D., de la Rasilla, M., Fortea, J. (in press). Dating of the hominid (*Homo neanderthalensis*) remains accumulation from El Sidrón cave (Piloña, Asturias, North Spain): an example of multi-methodological approach to the dating of Upper Pleistocene sites. *Archaeometry*.