Compressive marks from gravel substrate on vertebrate remains: a preliminary experimental study

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Abstract

Lakeshore sites such as Cerro de la Garita (Miocene of Teruel, Spain) and Senèze (Pliocene of Haute-Loire, France) yielded fossils with distinct puncture marks. These marks were described as "punctures surrounded by plastically deformed bone" by Pesquero (2006), Pesquero et al. (in press) and Fernández-Jalvo et al. (in press) who proposed that the marks were caused by compression or trampling on bones against coarse sediment grain when deposited in damp environments. A series of experiments was performed to test this hypothesis. Cow, red deer and fallow deer metapodials were compressed by applying a mechanical load on them against a gravel substrate under dry/damp environments. Results confirm that these characteristic puncture marks are associated with compression efforts on bones in wet environments.

Keywords: punctures, punctures with a ridge, wet environments, taphonomy, actualistic studies

1. Introduction

Trampling marks on the surfaces of fossil bones are common in palaeontological and archaeological sites (Haynes, 1980; 1983; Behrensmeyer et al., 1986; Fiorillo, 1989; Andrews, 1990; Lyman, 1994). Most trampling marks have been described as randomly orientated scratch marks on the surface of cranial and

postcranial fragments. These scratch marks are usually transverse to the long axis of shafts of long bones. Such marks are largely produced as the result of horizontal displacement when pressure is applied on the bone against the underlying substrate by animals trampling the bone. Experimental works have shown scratches produced by trampling may highly mimic those produced by human cutting (Andrews and Cook, 1985; Behrensmeyer et al., 1986; Olsen and Shipman, 1988; Domínguez-Rodrigo et al., 2009). This is especially relevant in palaeoanthropological sites when the identification of cut marks vs. trampling marks remain uncertain having strong repercussion on hominin technological evolution (McPherron et al., 2010; Domínguez-Rodrigo et al., 2010). Sediments in a dry substrate usually produce scratches by friction against the bone surfaces and, incidentally, some punctures by penetration of these grains in the bone cortex (Dominguez-Rodrigo et al. 2009, 2010). In lakeshore sites, such as Cerro de la Garita (Pesquero et al., 2013) and Senèze (Fernández-Jalvo et al., in press), some punctures are surrounded by plastically deformed bone, initially interpreted by these authors as caused by trampling against gravelous or coarser sediment immersed in water (Fig. 1). Experimental work described here monitors these observations and assesses how such punctures formed. The interest in correlating these type of punctures with damp conditions is to obtain a diagnostic criteria to infer flooding periods that may or may not be in agreement with the sedimentological traits and general environmental context indicating the homogeneity or mixtures during the site formation.

1.1. The sites

Cerro de la Garita is a reference site for Continental Euroasiatic Miocene mammalian fauna (MN unit 12 *sensu* Mein, 1990, 7 Mya). The palaeoenvironment was a palaeo-lakeshore along which fossil associations were formed by attritional mortality of large mammal species (Pesquero et al., in press). The lake site is highly calcareous and provided conditions that favoured the formation of delicate geodes of calcite crystals. The reference site of Senèze is a Pliocene maar (volcano crater) filled by a lake during the Villafranchian. The site is well-known as the biochronological international reference of MNQ 18, one of the most important fossil vertebrate collections of this period and the record of several holotypes. Senèze has yielded thousands of fossil vertebrates, several complete and partial skeletons, some in anatomical position. Taphonomic investigations show a high incidence of damage from falling blocks and trampling that mimics carnivore chewing to a great extent (Fernández-Jalvo et al., in press).

These two lake-shore sites yielded fossils bearing "punctures surrounded by plastically deformed bone" which were interpreted as the result of compression by trampling against sediment on bones while they were wet or immersed in water (Pesquero 2006; Pesquero et al., in press; Fernandez-Jalvo et al., in press). In order to understand processes by which taphonomic modifications are produced and the agents involved (Weigelt, 1927) direct observations by actualistic studies under controlled conditions are sometimes needed. We have experimentally reproduced depositional conditions to monitor the proposed hypothesis, and thus aid interpretation of the fossil record. In order to obtain maximum control during the experiments, we have used testing equipment that

repeats identical cycles using the same force and reduces the number of supplementary parameters that might influence the results.

1.2. Mechanical properties of bone

Depression marks are the response of bones under the action of an applied force or load. The mechanical properties of bone depend on its composition and structure. Bone is characterized by a complex hierarchical composite structure, consisting of approximately 60% inorganic phase (carbonated hydroxyapatite, CHAP), 30% organic phase (primarily fibrous polymer matrix of type I collagen) and 10% water (Zioupos, 2001; Nyman et al., 2006). Flexible fibers in bone are formed by intertwined collagen molecules which are bond together and to mineral components by non-collagenous proteins (Olszta et al., 2007; Thurner et al., 2009). In general, the mechanical properties of bone can be described in terms of stiffness (resistance to elastic deformation), strength (resistance to plastic deformation) and toughness (resistance to fracture). In this respect, several authors observe that bone behaves similarly to engineering materials (e.g. ceramic, metals, rocks, building materials) and, therefore, reacts to loading and fracture following the basic principles of mechanics (Currey, 1970; Burstein et al., 1975; Reilly and Burstein, 1974; Turner, 2006; Nyman et al., 2006; Agnew and Bolte, 2011, among others).

According to these mechanical principles, we can distinguish between *stress* (physical quantity defined as force per unit area average force per unit area) and *strain* (geometric measure of deformation representing a change in material

dimensions resulting from the application of a force). When stress is plotted against strain, a stress-strain curve is obtained (Fig. 2A). This curve can be divided into two distinct regions: the elastic region and plastic region, both connected by the yield point (Y).

The initial portion of the curve (straight line) (Fig. 2A), refers to the elasticity of the material, that is, the property of a material to return to its original dimensions when stress is removed. The slope of the linear region is called the *modulus of elasticity* (Young's Modulus). This modulus measures the inherent stiffness, which is related to a material's ability to resist plastic deformation. As stress increases, the material transforms from the elastic to plastic phase and begins to deform marking the yield point (Y), that is, the change in slope at the upper end of the linear region (Fig. 2A). As load is increased beyond Y, material becomes plastic (curve, Fig. 2A) and the material does not return to its original dimensions after removal of the stress. If the load is progressively increased, the material achieves its ultimate strength (TS=tensile stress or Maximum load, Fig. 2A) and beyond this point the material undergoes maximum deformation before ultimate fracture, that is how much load bone material can support prior to fracture. Finally, strain energy or toughness, which is a measure of a material's ability to absorb energy and resist fracture, is the area under the stress-strain curve and up to failure (shown in grey in Fig. 2A). On the basis of this area, materials that sustain little deformation before fracture are brittle materials (narrow strain energy area), whereas those that undergo significant deformation (wider strain area) are considered to be ductile materials (Fig. 2B).

2. Material and methods

The material used in the experiments includes a total of 35 modern bones of different animal sizes (Table 1). The actualistic study focuses on metapodials of cows (*Bos taurus*), fallow deer (*Dama dama*) and red deer (*Cervus elaphus*), which differ in their cortical thicknesses. These bones were collected in the field when already free of skin and meat and classified according to Behrensmeyer´s (1978) weathering stages. Thus, some bones still bear remains of grease (periosteum) without any cracking or flaking on their surfaces (stage 0), some show longitudinal cracking parallel to the fibre structure, or mosaic cracking of articular surfaces (stage 1), and some show flaking of their outer surface (stage 1-2). Where the sample was sufficiently large enough, it was possible to select bones that were at the same stage of weathering; as such, all fallow deer and red deer metapodials used in the experiments are at weathering stage 1. However, even in the case of deer skeletons, metapodials available for these experiments were not numerous. Due to present health laws, there was a higher constraint on bovids. Therefore, cow metapodials used here are of a weathering stage between 0 and 2, because the sample number was lower and it was impossible to select metapodials with a homogeneous weathering stage. We have considered different animal sizes to examine potential differences in the variability of dimensions of marks that may be linked to the relative density and thickness of the cortical bone. Body weight of individuals is estimated in accordance with the Rodriguez (1997) and Blumenschine (1986) classification: small sized=fallow deer; medium sized=red deer, and large sized=cows.

Bones were cleaned with a brush, to prevent any preparation based on a chemical reactive, boiling or mechanical tool, in order to avoid alteration of bone structure. All specimens selected were adult individuals to minimize differences due to age. All bones were photographed and analyzed under a binocular light microscope (Leica MZ 7.5 and real time-high resolution digital camera DFC450) to recognize macroscopic pre-existent modifications and distinguish them from those that may arise after the experimental series.

The study material was subdivided into non-hydrated and hydrated bones (Table 1). The first set of bones was always kept dry, whilst the second was immersed in water for an arbitrary time period of one month. Three different types of water were used: acidic (pH 4), neutral (pH 6.5) and alkaline (pH 10) which are the environmental natural values.

Experiments were conducted with a uni-axial compression machine Zwick/Roell Z5 (Fig. 3), and applied compression on the ventral and dorsal sides of the metapodials resting on a 3 cm thick of angular gravel substrate. Grain size range were measured between 2.9 and 8.8mm). Mechanical testing was conducted by applying a compression force of 2,500N (255kg $\cdot$m/s^2, 562lbf) on the central area of the shaft on the ventral and dorsal sides of the metapodials (Fig. 3). The tests ran arbitrary 50 cycles on each specimen and the data were processed using testXpert ® II software.

After compression testing, each specimen was examined again at 10x to 60x magnification under a binocular light microscope and photographed. All marks

recorded were counted both on the diaphysis and epiphysis of the side which had been in contact with the substrate. Frequency diagrams of marks on the bone surface were used to evaluate differences in function of the three categories of experiments.

Two non-parametric statistical tests were applied: *Mann-Whitney U* test comparing dry and hydrated bones and *Kruskal-Wallis* to test the tree species (or animal size) involved in the experiment; a *Turkey HSD test* compare similar size samples exposed to the three pH values used in the experiment.

3. Results

Bones that underwent the different experiments showed compressive marks caused by gravel grain that penetrated their surface when the bones were compressed against the substrate (Fig. 4A). The morphology of these marks (Figs. 4B and 4C), described as circular to oval depressions or punctures with different dimensions depending on grain size and the depth of penetration, roughly varying between 2 and 8mm. Typically, these marks have bowl-shaped cross-sections flat at the bottom, although some can be more angular. Punctures vary in depth; deeper marks may penetrate the compact bone to such an extent that the cortical bone collapses inwards. In areas where the compact tissue layer is thinner, as is the case with epiphyseal and metaphyseal regions, cancellous bone may be exposed (Fig. 4C). Some of these puncture marks are 'surrounded by plastically deformed bone' (hereinafter referred to as 'punctures with ridge' or P+R), whereas other punctures do not have a ridge

(hereinafter referred to as 'punctures without ridge' or P), as can be seen in Figure 4B.

Compression marks were counted based on the presence / absence of the distinct ridge surrounding the puncture edge (Table 2).

Experiments with non-hydrated bones, compressed against dry gravels, were performed on a sample of 7 bones (see Table 1). The most relevant result obtained from this experiment under dry conditions is the presence only of punctures without the characteristic ridge on the edge (Table 2).

Experiments with hydrated bones were performed on a sample of 28 bones (see Table 1) exposed to water with pH4, pH6.5 and pH 10 values. Puncture marks with a ridge (P+R) occurred under damp conditions, together with punctures without a ridge (P). The latter type of marks were always more abundant than the former (P+R) (Table 2). With regard to the rate of acidity, the number of marks with a ridge is higher on bones immersed in pH4 water than in bones immersed in pH6.5 water (although equal in fallow deer metapodials). Bones immersed in pH10 water are those bearing a lower number of marks with a ridge (P+R) surrounding the puncture edge (Table 2, Fig. 5).

We have not obtained accurate criteria to distinguish compression effects between soaked bones and immersed bones. This may in part be caused by the low number of bones available for these experiments. Puncture marks, with and without a ridge, were recorded on both the dorsal and ventral sides of metapodials. Those of cows and fallow deer have a similar number of punctures

on both sides, but red deer metapodials have a much higher number of punctures on the dorsal (65%) than the ventral (35%) side. With regard to animal size, there is no clear trend of an increase in number of punctures according to the thickness of the compact bone, at least on hydrated bones. This again is jeopardized by the low number of samples available for these experiments.

Punctures with a ridge (P+R) only appear on hydrated bones. These punctures are less abundant on bones exposed to pH10, gradually increasing with reduction of pH values. Such a pattern has not been observed in punctures without a ridge (P) which are more abundant on bones immersed in neutral waters (pH6.5, Fig.5, Table 2).

Comparison (through a t-student test) regarding the species type (cow, fallow and red deer) and the pH values (4, 6.5, 10) do not give any significant difference. A more specifically statistical treatment, a one-way ANOVA test, suggests that pH has an influence on the proportion of punctures with ridge (%P+R) ($p < 0.01$), which is significantly higher under acid conditions (Tukey's HSD Q, $p < 0.05$), whereas there are no relevant differences between neutral (pH=6,5) and alkaline (pH=10) conditions. In order to check if different species had any influence on the P+R percentage a Kruskal-Wallis test was performed but the results show that no significant differences exist in the P+R proportion between cow, fallow deer and red deer bones. The most relevant result obtained from this experiment is the total absence of punctures with a ridge in bones treated in dry conditions, a marked difference with respect to hydrated bones (Mann-Whitney U, $p < 0.0001$).

4. Discussion

In general terms, both collagen and mineral phases determine the mechanical properties of bone (Ritchie et al., 2009). The brittle hard mineral determines the strength and stiffness of the tissue and does not affect its strain-rate sensitivity (Kulin et al., 2011; Currey, 1988; Ritchie et al., 2009). However, ductile collagen, as a viscoelastic material, contributes to the rate-dependent fracture toughness of bone (Zioupos, 2001, Kulin et al., 2011; Wang et al., 2001; Buehler et al., 2008; Gautieri et al., 2009). Apart from the mineral and organic content, the biomechanical properties of bone are also related to water content, porosity (density), age and gender (Rincón et al., 2004, Koester et al., 2011).

4.1 Dry/wet bones: The effect of water

The most abundant marks recorded on bones from these experiments are puncture marks without a ridge (see Table 2). However, when bones were hydrated (both wet and immersed in water) a number of punctures surrounded by a ridge also occurred on bone surfaces (see Fig. 5). Punctures with a ridge (P+R) obtained in these experiments are highly similar to those identified in the Cerro de la Garita and Senèze sites. The absence of punctures with a ridge on dry bones and the fact that both fossil localities were lakeshores, suggest a link between these characteristic marks and damp environments, both permanently or periodically submerged.

As several authors have stated (Elliot and Robinson, 1957; Timmins and Wall, 1977; Nyman et al., 2006, among others), bone hydration significantly affects the mechanical behaviour of tissue, because the water is bound to both the surface of the collagen network and mineral phase. The amount of water within bone tissue affects its mechanical properties, mainly the viscoelastic behaviour of bone (Sasaki and Enyo, 1995). Water contributes to lowering Young's modulus (and thus stiffness, and increases recoverable strain (Dempster and Liddicoat, 1952; Evans and Lebow, 1951; Sedlin and Hirsch, 1966; Smith and Walmsley, 1959; Currey, 1965). In contrast, dry bone has different mechanical properties: a higher stiffness (higher Young's modulus), and much lower post-yield strain (Evans and Lebow, 1951), absorbing less energy before it fractures (Sedlin and Hirsch, 1966). Therefore, mechanical properties under identical compressive conditions differ between wet bones (that deform before breaking and are less stiff and dry bones (that reach their breaking point faster). In summary, wet bones are less stiff and brittle than dry bones.

According to Nyman et al. (2006) and our observations, plasticity increases in hydrated. Thus, the morphology of punctures in both immersed and soaked metapodials responds to the more plastic behaviour of wet bones, the tissue of which is deformed under compressive strain and retains this deformation. Such deformation is the ridge around punctures that we found on our experimental samples and on fossils of the Cerro de la Garita and Senèze sites.

4.2. Different environments: effects of acid/alkaline waters

Variation of water pH values may affect the chemical composition of bone, both its mineral and organic components. In this way, as the mineral component provides hardness and rigidity to the bone, when soaked in strong acid, the mineral dissolves (demineralizes), leaving most of the organic part (collagen) undamaged and the bone becomes a plastic material (White and Folkens, 2005; Figueiredo et al., 2011). In contrast, when bones are soaked in strong alkaline fluids, deproteinization occurs, and the bone becomes more brittle as only the mineral component remains (Collins et al., 2002; White and Folkens, 2005). Hence, the toughness of bone depends on its organic constituents, and its stiffness is due to its mineral content (Currey et al., 1996; Ziopus, 2001).

Several works have shown that the plasticity of bone depends on the protein phase. Burstein et al. (1975) found a decrease of Young's modulus in progressively demineralized compact bovine tibia that did not occur in the post-yield region, related to plastic deformation of collagen fibrils. In addition, Bowman et al. (1996) showed that the Young's modulus was much lower in completely demineralized compact bovine humeri than in untreated compact bone.

On the other hand, partially deproteinized compact bone has a Young's modulus similar to untreated bone but with much lower ultimate stress and strain (Catanese et al., 1999) whereas in partially deproteinized cancellous bone, the loss of organic material involves a significant decrease of the energy absorption ability, which results in brittle fracture (Fantner et al., 2004).

Our results are similar, as the maximum number of punctures with a ridge has been found on bones immersed in water with a 4pH value, which increases the plastic behaviour of bone. On the other hand, the lowest number of punctures with a ridge was found on bones exposed to alkaline waters, with partial loss of the organic matrix of the bone and a decrease in its toughness.

4.3. Morphological differences of the bone surface and cortical thickness

The topography (flatness/unevenness) of bone surfaces has influenced some of the results obtained from these experiments. The ventral and dorsal sides of fallow deer and cow metapodials used in the experiments have yielded a similar number of marks recorded on each side. In contrast, red deer metapodials (see Table 2) show a substantial difference between total number of marks recorded on dorsal (N=623) and on ventral (N=338) surfaces. The ventral surface of red deer metapodials have conspicuous salient lateral crests that in fallow deer are less prominent and are almost flat on cow metapodials. In order to obtain more accurate data concerning the shape of anatomical elements involved, these experiments should be extended to other anatomical elements. This was not, however, the aim of the present experiments; in order to gain conclusive results, from the preliminary results obtained here, the sample size should be increased.

4.4. Mimic marks

Marks obtained from compressive effects may share similarities with other taphonomic agents; for instance, this is the case of punctures made by carnivores. Indications that distinguish between these marks come, on the one hand, from the context of the site (e.g. other evidence of clear carnivore action) and, on the other, from puncture shape. Punctures caused by tooth marks are conical in shape, produced when tooth sharply pointed cusps penetrate the bone surface, whilst punctures by compression from stones or gravels are usually irregularly shaped and have a flat base and (although this ultimately depends on the grain morphology). These criteria were applied to the fossil collection from the site of Senèze (France), a volcanic maar where, before burial, bones were affected by falling blocks and compression against the sediment. The fact that bones were immersed in water also produced plastically deformed bone around punctures, and while irregularly shaped punctures with flat bases were abundant, no conical perforations were present (Fernández-Jalvo et al., in press). It is the case, however, that crocodile tooth marks may also produce punctures surrounded by plastically deformed bone, especially because chewing by crocodiles may also be produced under the water. Crocodile tooth marks have a characteristic bisected shape formed by the bicarinated tooth crown (Njau and Blumenschine, 2006; Baquedano et al., 2012) that does not appear in punctures arising from compression in sediment.

5. Conclusions

The experimental data presented here prove the hypothesis that bones immersed in water have plastically deformed bone around punctures (punctures

with a ridge). The experimentally obtained punctures are similar to marks observed in damp sites such as the fossil lake-shore sites of Cerro de la Garita and Senèze. These punctures with a ridge occurred together with non-deformed punctures on wet bones and those immersed in water, but they are absent on dry bones compressed against dry coarse sediment.

The present results also prove that bones immersed in water with different pH values showed disparities that could indicate different environmental conditions. Acidic situations could affect the chemical composition of bone producing demineralization of the inorganic component, leading to an increment of marks with the distinct ridge on the edge of punctures due to a higher plasticity of bone.

These preliminary results are especially relevant to past contexts, because compressive marks can be mistaken for other taphonomic processes, such as carnivore tooth marks, including those of crocodiles.

Diagnostic criteria to distinguish different taphonomic agents are needed and this experiment has shown a distinct characteristic modification associated to damp environments and gravelous ground surface that can show congruences/incongruences with the site sedimentology and help to discern mixtures due to reworking or time averaging.

Further work
As stated above, the preliminary characteristic of this work involves future developments that primarily include similar experiments with a considerably

larger sample size and additional anatomic elements and species. Additionally, it might be interesting to know if the number of marks would increase with longer immersion periods than one month, or what would be the morphological characteristics of those marks with well sorted/heterogeneous grain sizes and different categories of sediments.

Acknowledegements

References

Agnew, A.M., Bolte, J.H., 2011. Bone fracture. Biomechanics and Risk. In: Crowder, C., Stout, S. (Eds.), Bone histology: An anthropological Perspective. CRC Press, Boca Raton, Florida.

Andrews, P., 1990. Owls, caves and fossils. University of Chicago Press, Chicago.

Andrews, P.J., Cook, J., 1985. Natural modifications to bones in a temperate setting. Man (N.S.) 20, 675-691.

Baquedano, E., Domínguez-Rodrigo, M., Musiba, C., 2012. An experimental study of large mammal bone modification by crocodiles and its bearing on the interpretation of crocodile predation at FLK Zinj and FLK NN3. Journal of Archaeological Science 39(6), 1728-1737.

Behrensmeyer, A.K., 1978. Taphonomic and ecologic information on bone weathering. Paleobiology 4, 150-162.

Behrensmeyer, A.K., Gordon, K.D., Yanagi, G.T., 1986. Trampling as a cause of bone surface damage and pseudo-cutmarks. Nature 319, 768-771.

Blumenschine, R.J., 1986. Carcass consumption sequences and the archaeological distinction of scavenging and hunting. Journal of Human Evolution 15, 639-659.

Bowman, S.M., Zeind, J., Gibson L.J., Hayes, W.C., McMahon, T.A., 1996. The tensile behavior of demineralized bovine cortical bone. Journal of Biomechanics 29(11), 1497-1501.

Buehler, M.J. 2008. Nanomechanics of collagen fibrils under varying cross-link densities: atomistic and continuum studies. Journal of the Mechanical Behavior of Biomedical Materials 1(1), 59-67.

Burstein, A.H., Zika, J.M., Heiple, K.G., Klein, L., 1975. Contribution of collagen and mineral to the elastic-plastic properties of bone. Journal Bone and Joint Surgery 57, 956-961.

Catanese, J., Featherstone, J.D.B., Keaveny, T.M., 1999. Characterization of the mechanical and ultrastructural properties of heat-treated cortical bone for use as a bone substitute. Journal of Biomedical Materials Research 45, 327-336.

Collins, M.J., Nielsen-Marsh, C.M., Hiller, J., Smith. C.I., Roberts, J.P., 2002. The survival of organic matter in bone: A review. Archaeometry 44(3), 383-394.

Currey, J.D., 1965. Anelasticity in bone and echinoderm skeletons. Journal of Experimental Biology 43, 279-292.

Currey, JD., 1970. The mechanical properties of bone. Clinical Orthopaedics and Related Research 73, 210-231.

Currey, J.D., 1988. The effect of porosity and mineral content on the young's modulus of elasticity of compact bone. Journal of Biomechanics 21(2), 131-139.

Currey, J.D., Brear, K., Zioupos, P., 1996. The effects of aging and changes in mineral content in degrading the toughness of human femora. Journal of Biomechanics 29, 257-260.

Dempster, W.T., Liddicoat, R.T., 1952. Compact bone as a nonisotropic material. The American Journal of Anatomy 91(3), 331-362.

Domínguez-Rodrigo, M., Bunn, H.T., Pockering, T.R., 2010. Configurational approach to identifying the earliest hominin butchers. Proceedings of the National Academy of Sciences 107(49), 20929-20934.

Dominguez-Rodrigo, M., De Juan, S., Galan, A.B., 2009. A new protocol to differentiate trampling marks from butchery cut marks. Journal of Archaeological Science 36, 2643-2654.

Elliott, S.R., Robinson, R.A., 1957. The water content of bone. I. The mass of water, inorganic crystals, organic matrix, and CO_2 space components in a unit volume of the dog bone. Journal of Bone and Joint Surgery Am 39-A, 167-188.

Evans, F.G., Lebow, M., 1951. Regional differences in some of the physical properties of the human femur. Journal of Applied Physiology, 3 (9), 563-572.

Fantner, G.E., Birkendal, H., Kindt, J.H., Hassenkam, T., Weaver, J.C., Cutroni, J.A., Bosma, B.L., Bawzer, L., Finch, M.M., Cidade, G.A.G., Morse, D.E., Stucky, G.D., Hansma, P.K., 2004. Influence of the degradation of the organic matrix on the microscopic fracture behavior of trabecular bone. Bone 25, 1013-1022.

Fernández-Jalvo, Y., Valli, A.M.F., Marín-Monfort, M.D and Pesquero, M.D., in press. The taphonomy of Senèze. In: Delson, E., Faure, M., Guèrin, C. (Eds.), The site of Senèze. Book Series: Vertebrate Paleobiology and Paleonthropology. Springer, Dordrecht.

Figueiredo, M.M., Gamelas, J.A.F., Martins, A.G., 2011. Characterization of bone and bone-based graft materials using FTIR spectroscopy. In: Theophanides, T. (Ed.), Infrared Spectroscopy - Life and Biomedical Sciences. InTech, New York.

Fiorillo, A.R., 1989. An experimental study of trampling: implications for the fossil record. In: Bonnichsen, R., Sorg, M.H. (Eds.), Bone modification. University of Maine Center for the Study of the First Americans, Orono.

Gautieri, A., Buehler, M.J., Redaelli, A. 2009. Deformation rate controls elasticity and unfolding pathway of single tropocollagen molecules. Journal of the Mechanical Behavior of Biomedical Materials, 2(2), 130-137.

Haynes, G., 1980. Prey bones and predators: Potential ecologic information from analysis of bone sites. Ossa 7, 75-97.

Haynes, G., 1983. A guide for differentiating mammalian carnivore taxa responsible for gnaw damage to herbivore limb bones. Paleobiology 9(2), 164-172.

Koester, K.J., Barth, H.D., Ritchie, R.O., 2011. Effect of aging on the transverse toughness of human cortical bone: Evaluation by R-curves. Journal of Mechanical Behavior of Biomedical Materials 4, 1504-1513.

Kulin, R.M., Jiang, F., Vecchio, K.S. 2011. Effects of age and loading rate on equine cortical bone failure. Journal of the Mechanical Behavior of Biomedical Materials 4(1), 57-75.

Lyman, R. L. 1994. Vertebrate Taphonomy. Cambridge University Press, Cambridge.

McPherron, S. P., Alemseged, Z., Marean, C.W., Wynn, J.G., Reed, D., Geraads, D., Bobe, R., Béarat, H.A., 2010. Evidence for stone-tool-assisted consumption of animal tissues before 3.39 million years ago at Dikika, Ethiopia. Nature 466, 857-860.

Mein, P. 1990. Updating of MN Zones. Updating of MN zones. In: Lindsay, E.H., Fahlbusch, V., Mein, P. (Eds.), European Neogene Mammal Chronology, Nato ASI Ser., 180. Plenum Press, New York.

Njau, J.K., Blumenschine, R.J., 2006. A diagnosis of crocodile feeding traces on larger mammal bone, with fossil examples from the Plio-Pleistocene Olduvai Basin, Tanzania. Journal of Human Evolution 50, 142-162.

Nyman, J.S., Roy, A., Shen, X., Acuna, R.L., Tyler, J.H., Wang, X., 2006. The influence of water removal on the strength and toughness of cortical bone. Journal of Biomechanics 39(5), 931-938.

Olsen, S.L., Shipman, P., 1988. Surface modification on bone: trampling versus butchery. Journal of Archaeological Science 15, 535-553.

Olszta, M.J., Cheng, X., Jee, S.S., Kumar, R., Kim, Y., Kaufman, M.J., Douglas, E.P., Gower, L.B., 2007. Bone structure and formation: a new perspective. Materials Science and Engineering R 58, 77-116.

Pesquero, M.D., 2006. Tafonomía del yacimiento de vertebrados miocenos de Cerro de la Garita (Concud, Teruel). Ph.D Thesis, Complutense University of Madrid, Madrid.

Pesquero, M.D., Alcalá, L., Fernández-Jalvo, Y., in press. Taphonomy of the reference Miocene vertebrate mammal site of Cerro de la Garita (Concud, Teruel, Spain). Lethaia, DOI: 10.1111/let.12016.

Reilly, D.T., Burstein, A.H., 1974. The mechanical properties of cortical bone. Journal Bone and Joint Surgery 56, 1001-1022.

Rincón, E., Ros, A., Claramunt, R., Arranz, F., 2004. Caracterización mecánica del material óseo. Técnología y Desarrollo 2, 3-27.

Ritchie, R.O., Buehler, M.J., Hansma, P. 2009. "Plasticity and toughness in bone. Physics Today 62(6), 41-47.

Rodríguez, J., 1997. Análisis de la estructura de las comunidades de mamíferos del Pleistoceno de la Sierra de Atapuerca. Revisión de metodologías. Ph.D Thesis, Autonoma University of Madrid, Madrid.

Sasaki, N., Enyo, A., 1995. Viscoelastic properties of bone as a function of water content. Journal of Biomechanics 28(7), 809-815.

Sedlin, E.D., Hirsch, C. 1966. Factors affecting the determination of the physical properties of femoral cortical bone. Acta Orthopaedica Scandinavica 37(1), 29-48.

Smith, J.W., Walmsley, R., 1959. Factors affecting the elasticity of bone. Journal of Anatomy 93, 503-523.

Sultana, N. 2013. Biodegradable Polymer-Based Scaffolds for Bone Tissue Engineering. SpringerBriefs in Applied Sciences and Technology, Springer, Berlin Heidelberg.

Thurner, P.J., Lam. S., Weaver, J.C., Morse, D.E., Hansma, P.K. 2009. Localization of phosphorylated serine, osteopontin, and bone sialoprotein on bone fracture surfaces. Journal of Adhesion 85, 526-45.

Timmins, P.A., Wall, J.C., 1977. Bone water. Calcified Tissue Research 23, 1-5.

Turner, C.H., 2006. Bone strength: current concepts. Annals of the New York Academy of Sciences 1068, 429-446.

Wang, X., Bank, R.A., TeKoppele, J.M., Agrawal, C.M. 2001. The role of collagen in determining bone mechanical properties. Journal of Orthopaedic Research 19(6), 1021-1026.

White, T.D., Folkens, P.A., 2005. The Human Bone Manual. Academic Press, San Diego.

Weigelt, J., 1927. Resente Wirbeltierleichten und ihre Paläobiologische Bedeutung. Max Weg Verlag, Leipzig.

Zioupos, P., 2001. Ageing human bone: factors affecting its biomechanical properties and the role of collagen. Journal of Biomaterials Applications 15(3), 187-229.

Figure captions

Fig. 1. Punctures surrounded by plastically deformed bone. Left: bone fragment from Cerro de la Garita (Miocene of Teruel, Spain). Right: bone fragment from Senèze (Pliocene of Haute-Loire, France).

Fig. 2. A) Typical stress-strain diagram divided into two distinct deformation regions by yield point (Y): elastic deformation and plastic deformation. In the curve above the yield point, stress reaches a maximum load, which is called the tensile strength (T.S.). B) Schematic diagram showing the mechanical behaviour of apatite, collagen and compact bone. The stiffest and most brittle material is apatite which shows the smallest fracture energy whereas collagen is a ductile material that can endure a higher deformation. Bone represents an intermediate situation with both ductile and brittle qualities and some degree of yielding during loading as indicated by a slight curve in the elastic region. (Turner, 2006; Sultana, 2013).

Fig. 3. Clockwise from left: Equipment used to perform the experiments. Details of the data obtained and recorded by the testXpert® software. Experiment under immersion.
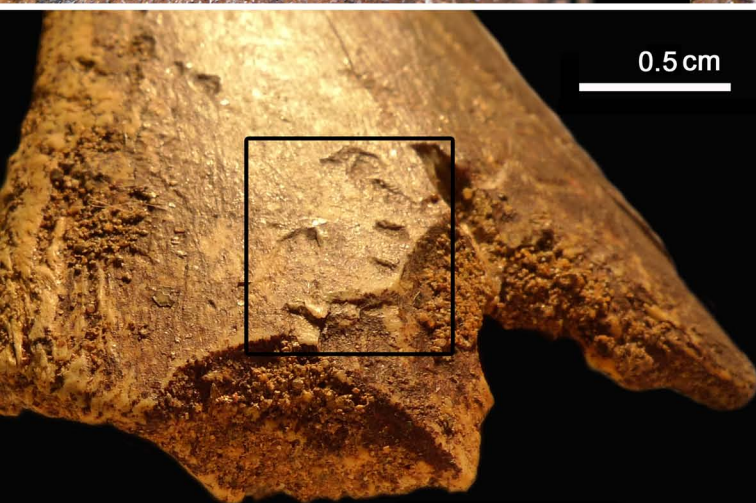
Fig. 4. A) Metapodials after the experiment showing gravel grains still attached to the surface (left) and after removing the gravels (right). B). Detail of depressions on a cortical bone shaft under the binocular light microscope. Above: depressions without a ridge; below: depressions with a ridge. C). Detail of compressive marks on articular ends exposing the underlying cancellous bone.
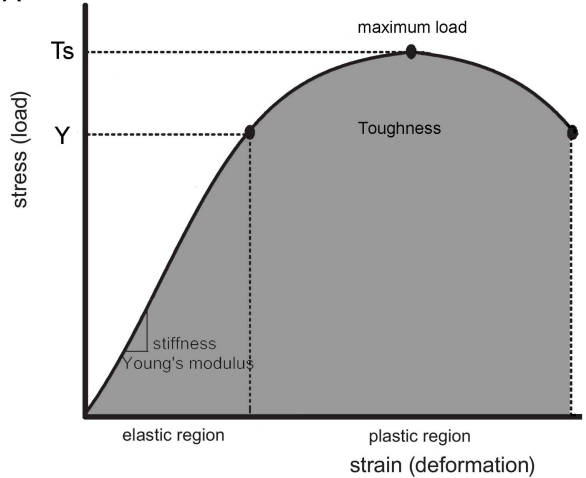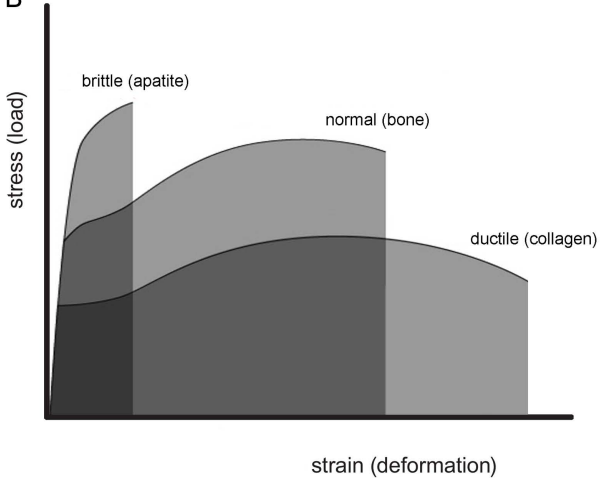
Fig. 5. Relative abundance of puncture marks recorded on each species under different experimental conditions
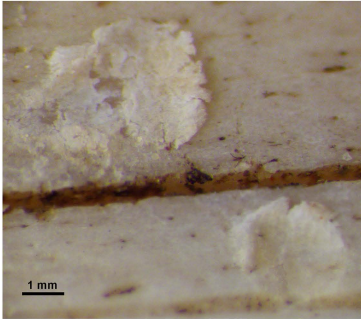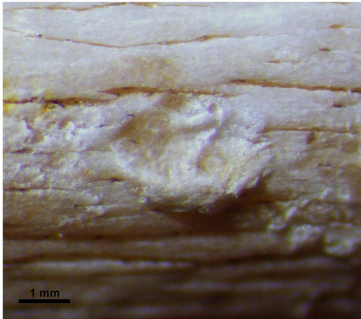
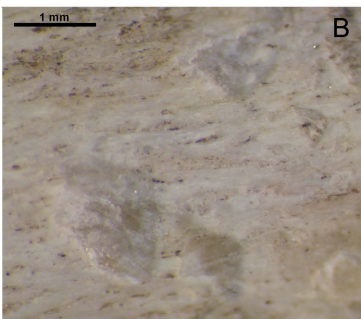**Table captions**

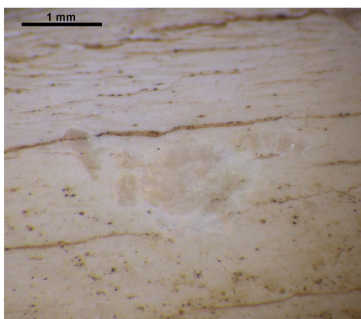**Table 1.** Number of bones available for the experiments: **Non-hydrated** bones of fallow and red deer and cow compressed against dry gravels. **Hydrated bones** (soaked and immersed in pH4/pH6.5/pH10 water) compressed against gravels.

Table 2. Total number of marks obtained from the experiments. n= number of bones; P= punctures (without ridge); P+R= punctures with ridge.

0,5 cm

0.5 cm

A — stress (load) vs strain (deformation)

Ts — maximum load

Y

Toughness

stiffness
Young's modulus

elastic region   plastic region

B — stress (load) vs strain (deformation)

brittle (apatite)

normal (bone)

ductile (collagen)
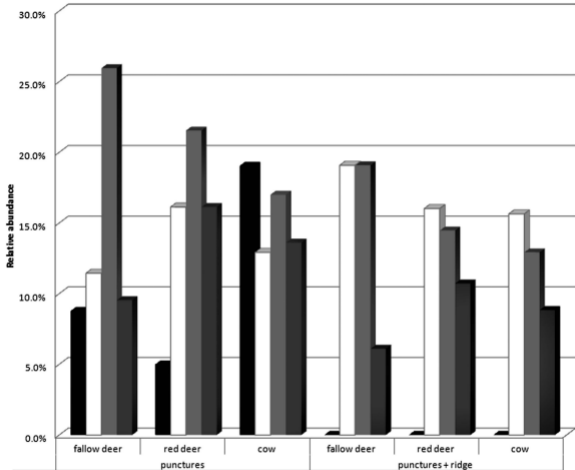
	fallow deer	red deer	cow	fallow deer	red deer	cow
		punctures			punctures + ridge	
■ DRY	8.8%	5.0%	19.0%	0.0%	0.0%	0.0%
□ pH4	11.5%	16.1%	12.9%	19.1%	16.0%	15.6%
■ pH6.5	26.0%	21.5%	17.0%	19.1%	14.5%	12.9%
■ pH10	9.5%	16.1%	13.6%	6.1%	10.7%	8.8%

	Non-hydrated	Hydrated bones		
		pH 4	pH 6.5	pH 10
Red deer	3	6	6	6
Fallow deer	3	2	3	2
Cow	1	1	1	1

Table 1. Number of bones available for the experiments:
Non-hydrated bones of fallow and red deer and cow compressed against dry gravels.
Hydrated bones (soaked and immersed in pH4/pH6.5/pH10 water) compressed against gravels.

	NUMBER MARKS								
	Fallow deer (n= 10)			Red deer (n= 21)			Cow (n=4)		
	n	P	P+R	n	P	P+R	n	P	P+R
DRY	3	23	0	3	48	0	1	28	0
pH4	2	30	50	6	155	154	1	19	23
pH6.5	3	68	50	6	207	139	1	25	19
pH10	2	25	16	6	155	103	1	20	13
Total	**10**	**146**	**116**	**21**	**565**	**396**	**4**	**92**	**55**
		262			961			147	

Table 2. Total number of marks obtained from the experiments. P= punctures (without ridge); P+R= punctures with ridge.