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=== REVIEW ===

BONE DIAGENESIS AND FTIR INDICES: A CORRELATION

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SUMMARY For the genetic analysis of ancient human remains to be done, the most appropriate bone has to be selected for the extraction procedure. The osseous tissue is already poor in DNA content, and the genetic material begins degradation from the moment of death. The burial conditions influence the diagenesis progress, affecting the composition and microstructure of the mineral component of the bone, the hydroxylapatite, and also the integrity of the organic fraction, which suffers chemical degradation and microbial attack. A complex interaction between the organic and the mineral part, and the impact of different burial site environments render remains with a variety of properties that affect the DNA availability. Using FTIR Spectroscopy (Fourier Transform InfraRed Spectroscopy), many of these variations in bone features can be interpreted, correlated, and used in various further methodological approaches. The crystallinity of the bone mineral and also its organic content can be characterized through FTIR. A higher level of crystal order corresponds to a more degraded bone, but a moderate adsorption of the DNA to the mineral crystallites can protect it from chemical decay, making the interpretation of FTIR spectra a complex and thoughtful procedure.

Keywords*:* ancient remains, crystallinity, diagenesis, FTIR.

Introduction

With the advent of state-of-the-art biological techniques, molecular bioarchaeology has made tremendous progress in the last decades (e.g. Margulies *et al.*, 2005) allowing the scientific world to decipher more and more secrets of the past. Of particular interest is learning the information encoded in the DNA of ancient skeletons. After specific nucleotide sequences are determined, further interpretation can be done, and understanding of the past takes a step forward. However, the

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genetic material is highly degraded and long enough, relevant fragments are difficult to obtain depending on the degree of diagenesis the bone has undergone (Weiner, 2010).

Bone organic and mineral degradation

DNA degradation commences right after death, through autolysis, as the endogenous enzymes from the lysosomes are released within the cells. All types of biomolecules are attacked by specific proteases, lipases, carbohydrases and nucleases, respectively. Some chemicals generated by this enzymatic digestion, such as peroxides and acids may further deteriorate other molecules, increasing damage (Brown and Brown, 2011). After the acute breakdown on the account of putrefaction is over (Collins *et al.*, 2002), remnant fragments of the macromolecules are then slowly suffering a chemical defacement. On one hand, *hydrolysis* manifests through the cleavage of β-N glycosidic bonds, leaving the DNA with an abasic site that will further lead to a nick in the strand. Cytosine and thymine are 20 times less liable than adenine and guanine to this peril (Brown and Brown, 2011, Lindahl, 1993). Furthermore, the packaging of the DNA *in vivo* has an effect on the size of initial fragmentation, affecting mitochondrial DNA differently than the nuclear DNA, as circular molecules are less accessible to nuclease activity (Collins *et al.*, 2002, Allentoft *et al.*, 2012). Hydrolysis also leads to miscoding lesions, where the bases are deaminated: cytosine turning into uracil that will complementary bind adenine instead of guanine, or adenine turning into hypoxanthine that will bind cytosine instead of thymine (Lindahl, 1993, Salamon *et al.*, 2005; Brown and Brown, 2011). Guanine, by deamination turns into xanthine that pairs with cytosine, so no mutation is induced, but xanthine will dislodge from the ribose, leading to the formation of an apurinic site (Lindahl, 1993). On the other hand, *oxidation* produces blocking lesions, scars obstructing the sliding of the DNA-polymerase on the polynucleotide chain, oxygen or its free radicals breaking chemical bonds within the bases or the ribose sugar (Brown and Brown, 2011). Water and oxygen are indeed detrimental to DNA conservation, as ancient spores, protected from excess water and kept anoxic by their shield seem to have a better preserved genetic material (Lindahl, 1993). Also, the Maillard reaction that links sugars and amino-acids, binding peptides to the polynucleotides, leads to blocking lesions. Other major participants at the degradation of the organic material are the ubiquitous microorganisms, whose secreted enzymes break molecules into smaller, absorbable ones (Brown and Brown, 2011). At first, bacteria are mostly of endogenous origin, from the intestinal tract, and after putrefaction is completed the soil microorganisms might continue the process if the environmental conditions are favorable (Jans *et al.*, 2004). Bell *et al.*(1996) considers the time between death and burial as a critical point in the diagenesis of a bone, greatly influencing its fate. Nevertheless, it is important to consider the fact that the DNA macromolecules are located in a complex medium, interacting with the surrounding molecules. Adsorbed to the Ca^{2+} ions in the mineral

hydroxylapatite via the negative phosphate groups, DNA is two times less affected by depurinization (Lindahl, 1993). A DNA molecule moderately adsorbed to the mineral is much more stable over time and less affected by degradation, but a bond too strong, owing to a higher surface area of the mineral crystallites, will heavily impair the DNA isolation rate (Götherström *et al.*, 2002).

The exact chemical reactions affecting the DNA after biological repair processes halt are mainly known, but the genetic material-to-whole bone ratio is infinitesimal, so a study of the other bone components is applied in order to assess the DNA availability.

The mature bone tissue is comprised of ~70% carbonate hydroxylapatite (CHA), 20% organic matter and 10% water. The organic proportion is made up of 90% type I collagen, the most abundant protein in the human body, with most of the rest 10% being osteocalcin (Buckley *et al*., 2008), the second most abundant protein in the bone, other non-collagenous proteins and proteoglycans, lipids, and of course, scarce amounts of DNA (Weiner, 2010). After death, the bone tissue becomes unstable, while part of the water evaporates, a proportion of the organic material is degraded and lost and the mineral fraction suffers extensive reorganization with an increase in crystallinity and changes in porosity (Weiner and Bar-Yosef, 1990; Collins *et al.*, 1995; Nielsen-Marsh and Hedges, 2000; Collins *et al.*, 2002; Hedges, 2002; Trueman *et al.*, 2004; Rogers *et al.*, 2010; Weiner, 2010; Brown and Brown, 2011; King *et al.*, 2011; Muller *et al*, 2011).

Bone diagenesis is a complex process that has numerous variables modeling it, so it is a rather difficult task to completely decipher all participations: microorganisms, temperature, local hydrology, oxygen content, soil organic content, geochemical properties and pH, and so on. A forensic study revealed that microbial attack in bone tissue occurs at around 3 months after death (Bell *et al*., 1996). Microbes digest bone collagen for nutritional purposes – along with the rest of the organic fraction – but neither their secreted enzymes nor they are able to penetrate the mineral lattice (Collins *et al.*, 1995). So a previous dissolution of the surrounding mineral layer is needed. Actually, the two operations are concurrent and limiting each other, in the first 50-100 days after death collagen cannot be attacked owing to an external layer of mineral not yet dissolved. The complete degradation of collagen, known as gelatinization, occurs at 70 °C if it is not mineralized, while in bone tissue this only happens at 150 °C, highlighting an interaction and an increase in stability (Collin *et al.*, 1995; Brown and Brown, 2011). The organic fraction existing in the composite lowers the mineral solubility, passivating its surface, because proteins with high acidic amino-acid content will bind Ca^{2+} atoms with their COO^- residues. Osteocalcin, also named the 'Gla protein' has a conserved central region of γ-carboxyglutamic acid residues representing the calcium binding region. This protein is linked to the mineral fraction tighter than collagen, and the only way to extract it is by dissolving the mineral itself (Buckley *et al.*, 2008; Weiner, 2010). Osteocalcin can be found in fossils, indicating its importance in stabilizing the bone (Berna *et al.*, 2004). The mineral and organic parts are intimately intertwined, a dissolved mineral phase exposing the collagen to degradation, while the

presence of proteins reduces the rate of mineral dissolution (Nielsen-Marsh and Hedges, 2000; Collins *et al.*, 2002). When all conditions for both processes are met the bone can eventually be totally dissolved (Nielsen-Marsh *et al.*, 2000).

Bacteria, fungi or cyanobacteria, in marine environments, greatly accelerate bone degradation by enzymatically degrading the collagen matrix, thus increasing porosity and crystallinity (Hedges, 2002). With higher porosity, water enters more easily into the bone structure, aiding recrystallinisation (Hedges, 2002). Electron microscopy imaging revealed 'microscopical focal destruction' tunnels of 5-10 μm in diameter as a result of fungi attack and hyper-mineralized bacterial remains in areas with small pores and thin channels (0,1- 2,0 μm) where bacteria have resided (Hackett, 1981). Wess *et al.* (2001) also observed hyper-mineralised zones with disoriented crystals in close vicinity to cells with very little organic substances, sustaining the idea that where bacteria have entered and consumed the organic component, the mineral phase also reacted, increasing crystallinity (Müller *et al.*, 2011).

Collagen can, too, be disintegrated by chemical degradation, under the action of water and oxygen from the environment. Chemical, uncatalyzed oxidation and hydrolysis are, however, much slower reactions compared to the enzymatic microbial digestion, but they affect the bone at a macroscopic scale, not only at small destruction foci (Hackett, 1981). A medium with extreme pH and temperature favors a chemical degradation, whilst a more neutral pH is needed for microbial growth (Collins *et al.*, 2002). Triple-helical collagen molecules bind together into structures of tropocollagen via hydrogen bonds and cross-links between lysine residues. Peptide bonds are hydrolysed, leaving nicks in the macromolecule. (Collins *et al.*, 1995). When the broken fragments are short enough that the hydrogen bonds holding them together are weaker than the thermal energy, the molecule breaks down (Brown and Brown, 2011). If the region where this is happening is open enough to the exterior, bacteria can absorb and digest the polypeptide fragments, but if the disintegration occurs in a niche surrounded by mineral, only small scale swelling and unraveling of the collagen occurs (Brown and Brown, 2011). This is because molecules bigger than the water molecule are not able to pass the mineral-protein composite, not even ethanol can, so any eventual bacterial enzymes are secluded (Collins *et al.*, 1995; Collins *et al.*, 1995; Nielsen-Marsh *et al.*, 2000; Brown and Brown, 2011). Cross-linking of collagen with large sized humics infiltrated into the bone from the surrounding soil or condensation with carbohydrates, leading to formation of Maillard products are reactions that help collagen preservation, as they hinder enzymatic digestion (Klinken and Hedges, 1995; Nicholson, 1998; Jans *et al.*, 2004).

The major bone fraction, the carbonate hydroxylapatite [$Ca_{10}(PO_4,CO_3)_6(OH)_2$] is a disordered version of the naturally occurring mineral hydroxylapatite, with some of the phosphates replaced by carbonate moieties. As the PO_4 group has a tetrahedral frame, and the CO_3 is a planar structure, once the natural composition is modified, the 'perfection' of the crystal lattice decreases (Weiner, 2010). Also, using electron microscopy, the size and shape of the crystallites in adult human bone have been determined: 50 x 25 x 2-4 nm and plate-shaped flakes, respectively (Weiner and Price,

1986). Such scanty dimensions determine a very high surface area: volume ratio, the specks having only 12-14 layers of atoms, meaning that a large proportion of them are actually at the surface, not bound to other atoms in a crystalline manner, so a high level of 'unsaturation' is observed. Thereupon, the bone mineral component is actually a pretty disordered structure that would naturally tend toward a thermodynamic equilibrium (Weiner and Price, 1986; Berna *et al.*, 2003; Salamon *et al.*, 2005; Weiner, 2010). After death, when the collagen matrix surrounding the HA crystallites gets degraded, the mineral is no longer caged and the course to equilibrium begins, increasing crystallinity (Collins *et al.*, 2002; Rogers *et al.*, 2010). This explains the hyper-mineralised zones aforementioned (Hackett, 1981, Wess *et al.*, 2001).

As the crystallites grow in dimension and get tighter and more orderly packed, their stability increases, namely their solubility decreases. Berna *et al.*, (2004) studied this property of the bone mineral in relation to phosphate concentration and pH. For fresh bone, on one side, and for a reference HA on the other, two parallel diagonal isotherms were plotted, and the area between them was referred to as a "recrystallization window". As the PO_4 concentration and pH get lower, dissolution occurs, i.e. the left hand side of the HA isotherm, while higher values of the variables induce solidification. A fresh bone subjected to an outset of diagenesis, at a certain – let's assume – *low* PO_4 concentration will have the mineral component dissolved when the surrounding pH drops below the corresponding value on the isotherm. At this point, the water closely enveloping the bone will have a local higher PO_4 concentration, shifting the terms in the "solid" area, thus leading to recrystallization. However, the dissolved mineral will now adhere to an already existing crystal, increasing its size and lattice order. Through this process the crystallinity of the bone mineral increases. Deep in the bone matrix, this water is entrapped in the natural pores, and will not be easily washed away. Still, when the water with diluted minerals from outside the remains is flushed and replaced with fresh water, recrystallization will no longer occur, but the dissolution might continue if the pH allows it. Berna *et al.* estimated that if the surrounding water is replaced every 120 days (seasonally), it would take approximately 25-50 years for 1 gram of bone to be totally disintegrated. In this respect, clay-type soils are extremely good at isolating the micro-environment. If simultaneous collagen digestion takes place, the bone is decomposed.

Each component of the bone composite goes through typical diagenesis paths, but the extent of each of these, and the precise succession leading to the state in which an ancient bone is recovered are governed by sundry factors, discussed below.

Environmental contribution

Different bones exhibit different levels of diagenesis, depending on the local burial site conditions rather than the age of the remains (Weiner and Bar-Yosef, 1990). This implies that the very many variables specific to each site influence the bone

degradation in different ways. (i) Temperature variations are adverse to bone conservation, as the remains found in caves with a constant temperature are well preserved (Weiner and Bar-Yosef, 1990; Collins *et al.*, 2002; Hedges, 2002; Jans *et al.*, 2004). The specific karstic high carbonate concentration in groundwater also enhances mineral deposition, along with a slightly alkaline pH (Wright and Schwarcz, 1996; Collins *et al.*, 2002). Very low temperatures, too, aid the survival of DNA, by slowing down any chemical degradation and halting the microbial growth. DNA fragments of unexpected lengths were successfully found in permafrost remains. On the other hand, high temperatures increase the yield of all chemical reactions and the growth of microorganisms, leading to degradation. (ii) Water is known to degrade the organic components of bone, and an arid environment seems to favor conservation, mostly by inhibition of bacterial development (Collins *et al.*, 2002; Götherström *et al*., 2002; Hedges, 2002; Trueman *et al.*, 2004). However, independently of the medium aridity, the collagen molecules are always hydrated (Collins *et al.*, 1995; Weiner, 2010) and the mineral micropores within the HA matrix are ever filled with a solution of water and mineral ions in equilibrium with the crystallite phase (Hedges, 2002; Berna *et al.*, 2004; Lebon *et al.*, 2010). Similarly, the DNA fragments necessarily have a layer of hydration that allows them a natural conformation, as a chemically dehydrated DNA would have a destroyed double helix structure and a much greater chance of damage (Lindahl, 1993). Excess water, in the case of waterlogged remains, leads to anoxia, blocking some bacterial degradation (Hedges, 2002). Ultimately, water fluctuations and an active soil hydrology are the hostile factors, rinsing the saturated mineral solution from the close vicinity of the bone composite, leading to bone dissolution (Wright and Schwarcz, 1996). (iii) The soil type of the archaeological site strongly marks the diagenesis of the remains, most references stating a clay-type soil as the best retainer. It is not permeable to water, so enclosed cavities around the bones would maintain the carbonate solution, not allowing rain water or other sources of fresh water to dissolve the microenvironment (Weiner and Bar-Yosef, 1990; Nielsen-Marsh and Hedges, 2000; Berna *et al.*, 2004). An organically rich soil, such as peat, guano inside a cave, or a midden becomes anoxic, the glut of organic material consuming all the available oxygen. However, the oxidation lowers the pH, favoring the mineral dissolution (Berna *et al.*, 2004; Jans *et al.*, 2004; Weiner, 2010). Certain chemical elements present in the soil might also influence the bacterial development, soils polluted with mercury, lead or copper maintaining a low microbial population (Jans *et al.*, 2004; Müller *et al.*, 2011). Ions such as sodium, magnesium, barium, iron, manganese, strontium can enter the HA lattice in the position of the calcium ion, lowering crystal neatness. Fluoride is an element that immensely influences the crystallinity of the mineral, by replacing the hydroxyl group, majorly increasing tightness of the lattice and implicitly, decreasing solubility. (Berna *et al.*, 2004; Lebon *et al.*, 2010). A high carbonate content in the soil will lower the crystallinity of the HA, as it disturbs the mineral lattice, but it will also be deposited on the bone surface and in the pores and cracks as calcite (Nielsen-Marsh and Hedges, 2000). (Fig. 1) shows an example of such diagenesis. Trueman *et al.* (2004) assessed the organic content of

bones deposited on the soil surface for as long as 30 years and the collagen lessened by 50% in the surface parts of the remains, and a 25% loss was measured in deeper cortical tissues, suggesting the importance of the condition in which a bone is deposited.

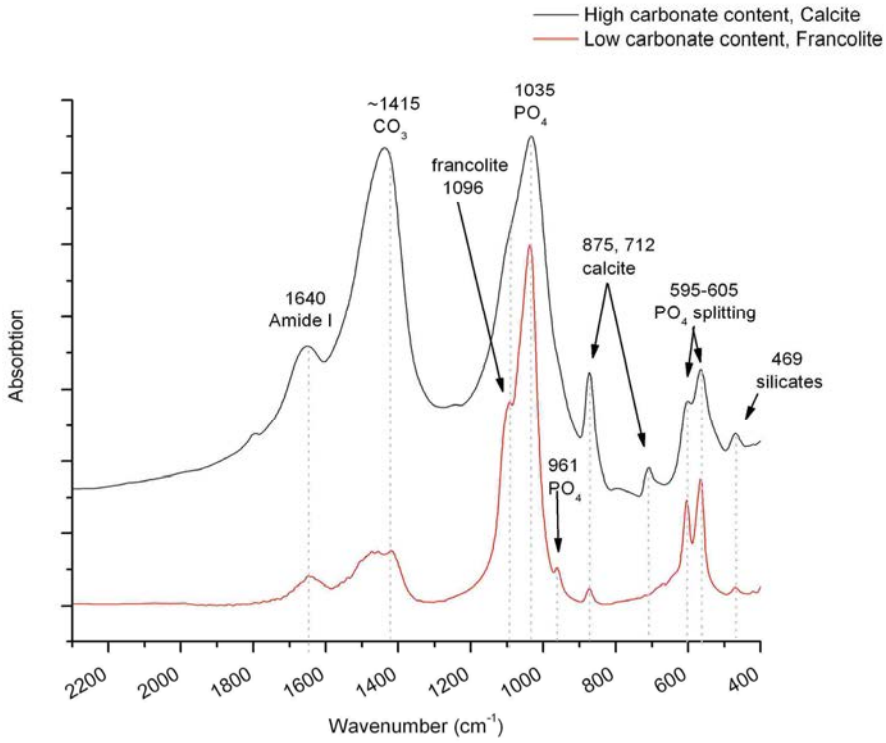

Figure 1. Examples of FTIR absorbtion spectra of bones showing different diagenesis patterns, according to the variables of the environment

The mineral and organic content, as well as the crystallite size, perfection and composition of the crystal lattice are features that are easily assessed with infrared spectroscopy. FTIR (Fourier Transform Infrared Spectroscopy) is a technique widely used in archaeology, together with methods like Raman spectroscopy, electron microscopy, X-Ray Diffraction. The main advantages of FTIR are the accessibility, low cost, relatively short preparation time and the small sample size. Also, FTIR can investigate amorphous samples, too, not only crystals, as XRD and Raman (Termine and Posner, 1966; Weiner, 2010; King *et al., 2011*). Therefore, an entire range of sub-domains can be studied using FTIR, not necessarily focused on ancient bio-molecules, or human remains, but also on non-biological directions, like pottery and cooking, clothing, a whole range of pigments (Damjanovi *et al.*, 2011; Karapanagiotis *et al.*, 2011; Rogerio-Candelera *et al.*, 2013).

FTIR indices used to assess bone diagenesis

FTIR is a spectroscopy technique that measures the absorption of infra-red light by the sample. The energy of the IR radiation can excite the chemical bonds, inducing stretchings, bendings, or vibrations between each pair of atoms or groups of atoms-functional groups (Coates, 2000). Every such absorption takes place at a given wavelength of the IR radiation, usually expressed as a wavenumber, the reciprocal of the wavelength, thus measured in cm^{-1}. Tables containing assignments of bond types and their proprietary frequencies can be found in the literature, with focus available on every desired domain, ranging from inorganic molecules to polymers, to any type of organic molecules (Bellamy, 1975; Socrates, 1994; Bryan *et al.*, 2007; Mello and Vidal, 2012). Therefore, when a sample is studied, the characteristics of the IR absorption spectra can lead to understanding the molecular shape of its chemical components.

The first FTIR index used to asses crystallinity in HA was the 'splitting function', an area-to-area ratio characteristic to the degenerating splitting of the 600 cm^{-1} phosphate ion antisymmetric bending frequency as the order of the atoms increased (Termine and Posner, 1966). Sometimes named 'crystallinity index'- CI, and sometimes 'infrared splitting factor'- IRSF, the division between the sum of absorbance values for the two peaks of the splitting and the value corresponding to the valley amid them (i.e. $(A_{595}+A_{605})/A_{588}$) encodes information regarding the degree of diagenesis a bone has undergone. As the organic phase of the bone is chemically or microbially degraded, the mineral lattice around it suffers dissolutions, mostly of the smaller, less stable crystallites, and recrystallizations on the already existing bigger crystals, according to the physicochemical surroundings as previously discussed. Nielsen-Marsh and Hedges (2000) state that the two processes, dissolution and recrystallization can together account for the gain in the IRSF value. This way, the crystallinity increases, and even if at the macroscopic level no differences can be seen, microscopic reorganization is traceable with the use of this parameter (Rogers *et al.*, 2010). A direct correlation between the IRSF and a mean crystal size was revealed by Truman *et al.* (2004), with a slight change in the shape of the crystallites as they matured, from plate-shaped to more needle-shaped: 163 x 28 x 5 nm (Berna *et al.*, 2004).

Several authors calculated the IRSF for modern bone in comparison to the archaeological bones they were studying at the moment, showing a definite increase with degradation: 2.7 *versus* over 3 (Müller *et al.*, 2011); less than 2.8 *versus* 2.7 - 3.4 (Trueman *et al.*, 2004); 3.4 *versus* 3,5 – 3,8 for one burial site and 3.1 – 4.6 for another one (Lebon *et al.*, 2010); 2.6 *versus* 3 (Nielsen-Marsh and Hedges, 2000). Berna *et al.* (2004) pointed out an even wider range of values: 2.6 - 3 for *in vivo* bones, up to 3.4 for bones slightly altered on the soil surface, up to 4.1 for fossil bones, and an IRSF of 7 was determined for highly altered fossils. For comparison, the IRSF for synthetic HA was 5.4. An explanation for why crystallinity in a bone can be higher than a standard HA will be given right away.

When the original chemical structure of the apatite is altered, the whole complex can get loosened, more soluble and less crystalline. This happens when the CO_3 partially replaces the PO_4 or the OH, or when the Ca is substituted by other atoms, such as Na or Mg**.** Figure 1 shows spectra of distinct types of diagenesis, the black plot corresponding to a bone deposited in a carbonated environment (massive carbonate peak at 1415 cm^{-1}): note the poor peak splitting at 595 – 605 cm^{-1}, meeting the explanation above. The reverse situation for HA is when the OH is replaced by a fluoride atom. The fluoroapatite $[Ca_{10}(PO_4)_6(F)_2]$ is actually packed tighter without the hydroxyl moiety, contracting the *a*-axis dimension, making the mineral much more insoluble, crystalline and pH resistant, with an IRSF of almost 7. In bone, the carbonate hydroxylapatite binds fluorine, turning into francolite $[Ca_{10}(PO_4,CO_3)_6(F)_2]$, a mineral with a specific infrared peak at 1096 cm^{-1}, easily recognized on the spectra by the specific 'francolite shoulder', near the main phosphate peak. The red plot in Fig. 1 is an example of a spectrum of a bone that absorbed fluorine, showing a distinctive francolite shoulder and a higher IRSF, as the split 600 cm^{-1} peak shows. King *et al.* (2011) found that burials from a slightly more hydrated linear area in an archaeological site had signs of francolite, as Greenwood and Earnshaw (1998) explained the high affinity of fluorine for water. Very old fossils, such as dinosaur fossils have IRSF values of almost 7 and high fluorine content. Otherwise, they could not have survived all this time (Berna *et al.*, 2004). Still, neither a high IRSF nor a short time of deposition correlate with a good organic conservation, as all the other factors described above outweigh the impact of chronological age (Weiner and Bar-Yosef, 1990). As an example, a mass Civil War grave failed to yield DNA for genetic analyses, while an older Saxon cemetery had interpretable DNA results (Hagelberg *et al.*, 1991). Salamon *et al.*, (2005) also failed to find any correlation between DNA amplification yield and the age of the sample, but Allentoft *et al.* (2012) argued that preservation is indeed a function of time, if all the other possible variables would be constant, as expected from pure physical chemistry data.

The 'carbonate content' measures the CO_3/PO_4 peak absorption ratio at 1415 and 1035 cm^{-1}, respectively. Wright and Schwartz (1996) established a good correlation between the C/P ratio and the weight% of CO_3^{2-} measured by CO_2 elimination by acid dissolution. C/P is a disputed parameter, mainly in the interest of performing isotopic labeling, any exogenous carbonate in the examined bone tissue yielding false results regarding age, migrations, dietary habits, climatology (King *et al.*, 2011). As for diagenesis, the C/P value simply shows the quantitative ratio between the anions. It might increase over time, if the surrounding carbonate content is high and thus turns the scale of the thermodynamic equilibrium against the HA natural tendency to eliminate CO_3. Such a case will also present with a specific calcite peak at 712 cm^{-1} (Fig. 1), if the calcite represents at least 3% of the bone mineral (Nielsen-Marsh and Hedges, 2000; Müller *et al.*, 2011). A lower C/P value indicates the loss of CO_3 through dissolution and the gain of PO_4 in the crystalline structure. Thereby, an average, modern C/P value in an ancient bone would indicate to both processes, a

deposition of calcite ($CaCO_3$) in and on the bone, with adsorption on the surface of the apatite crystals, but also to an increase in local crystallite perfection by reinstatement of PO_4 in the HA (Nielsen-Marsh and Hedges, 2000,***). Lebon *et al.* (2010) argued a fluctuating value of the C/P index in archaeological samples, on account of the many processes determining it, ranging from 0.15 to 0.35, while modern samples had a lower standard deviation, with an average value of the C/P of 0.28. However, the C/P index does raise some issues because of the organic C - H vibrations that absorb IR energy in the same wavelength as the CO_3 functionality, leading to an overestimation of carbonate along with the organic matrix content (Truman *et al.*, 2004). In Fig. 1 the more carbonated spectrum also has a high Amide I peak (Amide I is the C=O bond in the polypeptide chain), supporting the preceding observation.

The 'collagen content' CC is the index measuring the proteic preservation of the bone matrix, calculated from the peak absorption values of Amide I and PO_4, A_{1640}/A_{1035}. Values of 0.2 correspond to 15 wt% and of 0.8 to 30 wt% of organic material in bone (Truman *et al.*, 2004). The higher the protein signature is, the more organic fraction survived the passing of time in a specific bone. Nielsen-Marsh and Hedges (2000) state a correlation between the CC and the IRSF, as the loss of organic material leads to changes in the mineral microstructure by recrystallization, correlation not attained by Lebon *et al* (2010), probably because the lot investigated by them had some other factors influencing the indices, like fluoride.

Lebon *et al.* (2010) used an additional absorption value ratio to verify the increase in crystallinity, A_{1030}/A_{1020}. The 1030 cm^{-1} indicates phosphates in stoichiometric apatites (i.e. all six positions in $[Ca_{10}(PO_4)_6(OH)_2]$ are occupied by PO_4) and 1020 cm^{-1} is indicative of non-stoichiometric apatites containing CO_3^{2-} and/or HPO_4^{2-}. A direct correlation between this index and the IRSF was observed in a wide range of diagenetically altered bones, demonstrating the possibility to measure the crystallinity of fossil bones using either of the indices, especially when using micro-FTIR, where the IRSF 600 cm^{-1} is outside the detection range. A high non-stoichiometry correlated with high CC, as the non-perfect crystallites were protected from dissolution by the organic matrix (Lebon *et al.*, 2010).

A novel index proposed by Lebon *et al.* (2010) is the position shift of the ν_1PO_4 band at 960 cm^{-1} toward 963 cm^{-1}, linearly correlated with carbonate loss and with an increase of the crystal lattice perfection, as foreign ions are excluded. This index seems to be more sensitive to carbonate loss and lattice strain changes than the IRSF, which mainly refers to crystal size. Some samples might have this peak shifted above 963 cm^{-1}, but only with an accompanying high fluoride content that renders a more ordered atomic arrangement in the fluoroapatite compared to HA. Apatites doped with ions such as Ba^{2+}, Mn^{2+}, Sr^{2+} have the ν_1PO_4 peak shifted below 960 cm^{-1}, so this index could be used to detect ionic substitutions in the mineral lattice during diagenesis (Thomas *et al.*, 2007).

Conclusions

Bone diagenesis affects both fractions of the bone composite, the mineral part going through successive dissolutions and recrystallizations while the organic fraction suffers chemical degradation and microbial digestion. The two are deeply intertwined, influencing the extent to which the external factors can modify them. DNA can not be effectively observed in ancient bones, so its estimation must be done based on the other, much accessible components. The FTIR spectra of the bones examined give values for indices such as infrared splitting factor [(565+605)/588], carbonate-to-phosphate ratio (1415/1035), collagen content (1640/1035), stoichiometry of crystallites (1030/1020) and the perfection of lattice (the shifting of the ν_1PO_4 peak), and can also 'see' other important elements, such as the presence of fluoroapatite, or carbonate. Interpretation of all the accessible information from FTIR spectra of the bones at hand helps the molecular geneticist choose the most appropriate bone for extraction of DNA, in order to have the best DNA yield possible.

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