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ESTADO DA ARTE

The micro-analysis of human burned bones: some remarks

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ABSTRACT

The interdisciplinary research of burned bones is focused in this paper by presenting and discussing some methods that can assist the bioanthropologist in the analysis of this kind of remains. In particular, some techniques based on the histological structure of bone and on its molecular composition allow new ways of identifying burned human bone and of determining some aspects of the biological and ontological profile of an individual. A brief summary of those techniques is thus here presented.

Keywords: biological anthropology; forensic anthropology; bioarchaeology; burned human bone identification; stable isotopes; radiocarbon dating

RESUMO

A investigação de ossos queimados baseada numa abordagem interdisciplinar é focada no presente artigo a partir da apresentação e discussão de alguns métodos que podem ser úteis ao bioantropólogo envolvido na análise deste tipo de restos humanos. Em particular, algumas técnicas recentemente desenvolvidas e baseadas na histologia do osso e na sua composição molecular podem contribuir para a identificação de osso humano queimado e para a determinação de alguns aspetos relacionados com o perfil biológico e ontológico do indivíduo. Uma breve descrição dessas técnicas é aqui apresentada.

Palavras-chave: antropologia biológica; antropologia forense; bioarqueologia; identificação de ossos humanos queimados; isótopos estáveis; datação por radiocarbono

Introduction

Although some progress has been made in recent years, nowadays burned bones still represent one of the main challenges that bioanthropologists have to face whilst analyzing human skeletons. This is so because of the high fragmentation and other heat-related changes affecting bones that inevitably impair our ability to retrieve information from them. As a result, a critique of the bioanthropological methods that are conventionally used is required whenever burned bones are analyzed. This has been done to some degree in the past (Van Vark *et al.*, 1974; 1975; Buikstra and Swegle, 1989; McKinley, 1989; Duday *et al.*, 2000; Thompson, 2002, 2005; Ubelaker, 2009; Gonçalves *et al.*, 2011a; Gonçalves *et al.*, 2011b; Gonçalves, 2012). However, bioanthropologists are sometimes unaware of the potential of other approaches regarding the analysis of burned bones which can unquestionably increase the amount of information drawn from this sort of human remains. Therefore, a summation of those potentialities is here presented with the aim of assisting the bioanthropologist in his task of, not only analyzing bones, but also of maximizing the retrieval of data from them by resorting to other than bioanthropological analyses.

Identifying burned bone

The determination of whether or not the bone is burned is a very important issue. This assessment influences our own ability to identify human bone fragments and sets the bioanthropological methods that are to be used in the analysis. However, assessing if bone was affected by a heat-source is not a straightforward procedure. Although some macroscopic heat-induced changes – colour, fractures, dimension and warping – may assist us in such determination (revised by: Duday *et al.*, 2000; McKinley and Bond, 2001; Silva, 2007), these do not always allow for a conclusive judgment because other taphonomic and pathological factors can mimic them. For instance, colour changes can occur in bone as a result of soil discolouration or sun exposure (Shahack-Gross *et al.*, 1997; Buikstra and Ubelaker, 1994). Fractures can be produced by several agents such as the ones related to bioturbation or to weathering (Buikstra and Ubelaker, 1994). Post-depositional changes in size can also occur (Piepenbrink, 1986), although probably not in such a significant degree as the dimensional alterations witnessed in burned bones. Warped bones can be caused by several pathologies like rickets, osteomalacia, Paget's disease (Mays, 2008) or congenital syphilis (Ortner, 2008). Therefore, the recognition of bone affected by heat is not always an easy task.

Some researchers have turned their attention to alternative ways of identifying burned bones. For this purpose, the potential of the crystallinity index (CI) – or splitting factor – has been investigated intensively in the last years (Stiner *et al.*, 1995; Koon *et al.*, 2003; Munro *et al.*, 2007; Olsen *et al.*, 2008; Thompson *et al.*, 2009). The CI measures the order of the crystal structure and composition within bone. The premise behind its use is that the CI increases as crystals become larger and more ordered (Trueman *et al.*, 2008). This naturally occurs at a gradual rate after death but this process is fastened by some diagenetic pathways (Thompson *et al.*, 2009) and an exponential acceleration is furthermore promoted by weathering and heat (e.g.: Stiner *et al.*, 1995; Olsen *et al.*, 2008). Although taphonomy indeed interferes with the CI values, this approach has nonetheless good potential for the identification of burned bones – as long as fossilized bones and weathered bones are left out of this kind of analyses. This is particularly so if the CI values are interpreted in association with the carbonate to phosphate ratio according to Thompson *et al.* (2009). Even though the precise temperature at which bone was submitted to cannot be determined, these authors state that a differentiation between low and high temperature burnings can be established. The CI is assessed by X-ray diffraction (XRD), small-angle X-ray scattering (SAXS) or Fourier Transform Infrared Spectroscopy (FTIR) analyses (Shipman *et al.*, 1984; Hiller *et al.*, 2003; Thompson *et al.*, 2009).

A different approach has been taken by other researchers who have used light microscopy to observe the histological features of sectioned bone. Hanson and Cain (2007) used the microscopic internal structure to differentiate burned from unburned bones of sheep. Although no differences could be found between unburned bones and bones burned at low temperatures, some changes were documented as being the result of burning at medium/high or at high temperatures. Specifically, cracks extending outwards from the haversian canals were present in the first case while a loss of histological structures was observed in the second one. In an investigation with bone specimens from modern cattle, Harbeck *et al.* (2011) found that heat-induced changes – composed of small fissures – first appear in bone heated at 200^o C, while a more marked deterioration of the histological structure was documented at 500^o C. Corroborating the observations made by Hanson and Cain (2007), the structural elements were no longer distinguishable in bone sections heated at 800^o C. Unfortunately, these guidelines are not straightforward. Contrastingly, Cattaneo *et al.* (1999) were still able to discern the haversian systems in human and non-human bone heated at $800\text{-}1200^o$ C under the light microscope. This finding had already been documented by Holden *et al.* (1995) while resorting to the scanning electron microscope (SEM) to analyse human bone. Therefore, although a distinction between burned and unburned bone is feasible by looking at the histological structure, a more specific determination of the maximum temperature at which the burning occurred is still problematic. The differentiation between low and high temperature burnings outlined by Hanson and Cain (2007) seems to be a more conservative and reliable approach while using this method.

Shipman *et al.* (1984) and Nicholson (1993) also analysed microscopic features but focussed on the morphology of bone surface. This was done through SEM analysis. Although neither one have included unburned bone in their analyses, both have presented descriptions for various heating stages. In sum, an undulating surface with observable vascular canals was present at temperatures lower than 200^o C; at approximately 300^o C, bone surface presented a glassy appearance; then, bone acquired a frothy appearance when heated at 400-700^o C; finally, melting and coalescence of particles into larger structures with very variable shapes occurred at temperatures above 800^o C. The observations of both authors seem to be somewhat uniform thus possibly having good potential to infer more specific temperature determinations. However, weathering and fossilization are once again misleading agents because they can mimic heat-induced changes (Nicholson, 1993; Hanson and Cain, 2007). As a result, it also seems safer in this case to limit ourselves to distinguish bones heated at lower temperatures from bones heated at higher temperatures.

Identifying human burned bone

Heat-induced changes sometimes lead to the impossibility of determining macroscopically if an assemblage of osteological remains is human or not. Fragmentation may be so extreme that no recognizable features are preserved. Therefore, some alternative methods have been investigated in the last few years. Cuijpers *et al.* (2006) stated that, at least for the primary diaphyseal bone structure, the difference between humans – which is essentially composed of lamellar bone – and some large mammals – which is composed of fibro-lamellar bone – is useful to make a distinction. The authors argue that, since no significant changes in bone microstructure occur at temperatures up to 800^o C, the observation of its features is still achievable. Nonetheless, this statement still needs further validation because their research was carried out only on unburned bones. In addition, the authors state that bone structure alone may be sometimes misleading because fibro-lamellar bone is also present in humans during growing spurts or during fracture repairs so caution is required when using this approach.

Cattaneo *et al.* (2009) also used the histological structure to differentiate humans from non-humans. They chose to analyse osteons both metrically and morphologically in order to assess if these approaches were of some use in this matter. Indeed, the metric analysis allowed for a correct classification of all specimens by using discriminant function analysis specifically developed for this purpose, although the predicted correct classification was calculated to be of only 79%. As for the morphological assessment – in which any bone section presenting irregularly shaped osteons set in parallel rows and the presence of plexiform bone was determined to be non-human – this procedure was not as successful. Two to four cases were misclassified by two observers.

Another approach was taken by Cattaneo *et al.* (1994) who have attempted the immunological detection of human albumin in 31 archaeological cremations. Their success rate was of 26% and the authors concluded at this point that albumin can survive to cremation around 300^{o} C on account of occasional incomplete cremation or thanks to the insulating effect of soft tissues on bones. In another investigation, Cattaneo *et al.* (2009) were still successful at detecting this protein in burned remains subjected to temperatures ranging from 800 to 1200^{o} C, but this was so in only 4 out of 9 cases. This demonstrated that although burning events are very destructive of human albumin, it may still be preserved in some cases.

Although having its own problems, DNA analysis of burned bones also seems to be of some value. Besides allowing for the identification of human remains, DNA may sometimes be the only way to achieve the identification of individuals or can also help on the biological and demographic profiling of paleo-populations. DNA retrieval is hardly achievable for burned bones and teeth because genetic material is very sensitive to heat thus preserving badly (Ye *et al.*, 2004). Nonetheless, some researchers have been successful in doing so (Brown *et al.*, 1995; Sweet and Sweet, 1995; Williams *et al.*, 2004; Ye *et al.*, 2004). Wurmb-Schwark *et al.* (2004) found out that the DNA retrieved from burned remains did not match a buccal swab taken prior to cremation. The contamination problems surrounding this kind of procedure were therefore highlighted. Harbeck *et al.* (2011) stated that the analyses of remains from modern crematoria and from archaeological context are not advisable because the handling of such remains can lead to contamination. Nonetheless, they were able to retrieve DNA from modern cattle tibiae subjected to temperatures up to 700^{o} C. The authors stated that the duration of heat exposure has an important role in the preservation of genetic material. DNA analysis is then one more alternative to consider despite the limitations abovementioned.

Beckett *et al.* (2011) have proposed another method of identifying human bones. This one is based on the lattice parameters of bone mineral crystals. According to them, these present significant inter-species variation and can therefore be used to distinguish human from non-human bone through X-ray diffraction analysis. The investigation indicated that this method can be adopted when dealing with bones heated to temperatures up to 600^{o} C and 1400^{o} C. Nonetheless, these results were obtained on modern specimens and its value for archaeological materials is still unknown since diagenetic changes may interfere with the lattice parameters (Hedges, 2002). In addition, further validation of this method is required to confirm its reliability.

Documenting the ontological profile

Stable isotopic analyses may provide with important information regarding the ontological profile of an individual. For that purpose, carbon (C) and nitrogen (N) light elements give us some indication of the diet while oxygen (O) of bone apatite is useful to help determining the geographic origin of someone. In addition, strontium (Sr) gives clues about the geographic origin and the migrant movements. Harbeck *et al.* (2011) concluded that the latter is unaltered even at temperatures of 1000^o C, so it can be examined in burned skeletal remains. This had already been demonstrated by Grupe and Hummel (1991). In contrast, the remaining elements stay only unchanged at temperatures lower than 200^o C (Harbeck *et al.*, 2011) which somewhat corroborates the previous conclusions presented by Deniro *et al.* (1985). Harbeck *et al.* (2011) thus state that no reliable biological signal should therefore be expected for specimens heated at higher temperatures and that bone colourations including black, grey or white are indicative of material that is unfit for these kinds of analyses.

A negative correlation between temperature and the δ^{13} C values was recorded by Harbeck *et al.* (2011). This was also observed by Deniro *et al.* (1985) but nothing of the sort was documented by Schurr *et al.* (2008). As for the δ^{15}N, both of the latter have found an enrichment progression according to increasing temperature which is in contrast to what Harbeck *et al.* (2011) have found. Differences in sampling and experimental conditions may eventually explain these contrasting results. With the mentioned exception of Sr, the potential of stable isotopic analyses is apparently very limited for the inspection of burned bones. If the 200^o C hurdle is indeed confirmed as an indicator of the usefulness of these analyses, then this means that many of the burned skeletal remains handled by bioanthropologists cannot be subject to such examinations. This is especially true for archaeological cremations since most remains present charring or calcination.

Besides knowing where an individual lived in, it is also important to know when that occurred. The dating of burned bones is nowadays a reality by using the structural carbonate from the mineral fraction of bone instead of using the collagen fraction. This is possible because carbonate ions are incorporated into the inorganic bone matrix in living organisms as a substitution for phosphate in the crystal lattice (Lanting *et al.*, 2001; Olsen *et al.*, 2008). Lanting *et al.* (2001) found that this structural carbonate could be used to successfully date burned bones by resorting to AMS dating techniques which require small amounts of bone (2 g). The success of such procedure however, is influenced by temperature and by re-crystallization of the mineral matrix as was demonstrated by Olsen *et al.* (2008). These authors found a difference of approximately 160 ^{14}C years (sd = 34) between charred and calcined bones from the same individual of Late-Neolithic provenance. The bones heated at the lower temperatures – the charred ones – yielded slightly younger ages. Therefore, sampling should preferentially focus on calcined white bone.

Conclusion

The bioanthropological analysis of burned human skeletal remains may sometimes lead to a very limited amount of information due to the fragmentation and heat-induced changes that this kind of material often displays. A wider approach that does not rely only on the gross observation of bones may allow obtaining a better knowledge of the targeted individual.

Some of the procedures that have been described may be expensive or hard to access to. Light microscopes or SEM are now more common, but the other equipments mentioned in this paper are not always as easy to reach. Nonetheless, such analytical approaches may prove priceless in some cases.

The first concern when dealing with burned bones is to determine if these are indeed burned – and to what degree – and if they are actually human. Other assessments regarding the biological and ontological profiles should be achieved only after that confirmation is carried out because the selection of analytical methods depends on it.

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