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Ancient DNA: the quest for the best

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It is the dream of all researchers working with ancient DNA to identify prior to DNA extraction from bone the specimens or specific zones within them that contain the most and highest proportion of endogenous DNA. Since it impacts the sacrifice of precious ancient specimens and the financial support needed for the analyses, the question is of high importance to the scientific field of paleogenomics. The “Holy Grail” of paleogenomics was reached when Cristina Gamba and collaborators discovered that it was in the petrosal part of the temporal bone, the densest part of the mammalian skeleton, where DNA is exceptionally well preserved. As a consequence, osteological collections experienced a rush from paleogenomicists to “harvest” these precious bone parts. In this issue of Molecular Ecology Resources, Alberti and colleagues (2018) describe the discovery of another promising source of relatively well preserved endogenous DNA, the outermost layer of cortical bone, that they had identified through computed tomography (CT scans). These bones being larger and more abundant than petrous bones, this discovery increases markedly the source material for high quality paleogenomic studies and releases the pressure on osteological collections.

KEYWORDS

Ancient DNA, DNA preservation, taphonomy, bone

Paleogenomics, or the analysis of DNA preserved in biological material from the past, mainly bones, contributes greatly to the study of the recent evolutionary history of humans, human and animal migrations, domestication processes, past population dynamics, and the impact of climate change on past biodiversity, to name just a few. The primary source of these studies, however, is limited in number. Moreover, paleogenomic analyses being costly, and the failure rate due to insufficient DNA preservation high, researchers in the field were seeking for the “Holy Grail” since the initial proposition of differential DNA preservation in molecular preservation niches (Geigl 2002). Empirical considerations and observations motivated ancient DNA researchers to privilege the most compact bones for their studies. Gamba et al. (2014) pushed this logic to its ultimate limit and discovered that the most compact bone of the mammalian skeleton, the petrosal part of the temporal bone, is by far the best source of endogenous DNA from ancient bones. Since then, our scientific community could verify the truth and robustness of this observation that has transformed the field of paleogenomics to a point that a joke dates studies before 2014 to BP (before petrous).

In the present study, Alberti et al. (2018) used CT scans to identify the most compact parts of bones. This allowed them to refine the identification of the zones within the petrosal to be the most compact and favorable for DNA preservation. Most importantly, however, they identified a part of ancient

bones rich in DNA that so far has not only been neglected by paleogeneticists but has even been removed and discarded to minimize contamination: the outermost layer of cortical bone. Here, endogenous DNA is shown to be often (significantly in about half of the cases) much better preserved and less diluted in environmental DNA than in trabecular bone (Fig. 1). Even though it does not reach the DNA preservation levels of the petrosal, cortical bone is so much more abundant in the fossil record and in osteological collections that this observation solves a growing problem in the field: the access to fossil material in which DNA is well preserved.

To contextualize the problem of DNA preservation and the slow speed of progress of the field, it is necessary to take into account the great variability of DNA preservation in ancient bones which is responsible for differences in the efficiency of DNA extraction and the ratio of endogenous to environmental DNA. The variability in DNA preservation is such that even petrosal bones can sometimes contain only traces of endogenous DNA ((Pinhasi, et al. 2015); Fig. 1, our unpublished observations). Therefore, to be useful, quantitative studies must include many samples of various origins, periods and taphonomic contexts, but such studies are rare (e.g., (Pruvost, et al. 2007; Pruvost, et al. 2008)).

How does the compactness of bone favor DNA preservation? To understand this phenomenon, one has to consider all the biological and physicochemical parameters acting during bone diagenesis. Post-mortem DNA degradation is shaped by two distinct phases that determine the outcome. The first decay phase corresponds to the enzymatic degradation of most of the organic material involving autolysis and putrefaction. This phase of early diagenesis is most influential on the quantity and size of the DNA molecules that will persist and is probably responsible for the absence of a correlation between the age of the sample and the average size of preserved DNA molecules (Sawyer, et al. 2012; Kistler, et al. 2017). This first phase is likely to be subject to a great deal of variability but is a black box since one cannot reconstruct it ex-post. During the second decay phase, non-enzymatic chemical degradation seems to correlate with the thermal age of the samples, and in particular the deamination of cytosines at the ends of the DNA molecules (Sawyer, et al. 2012; Kistler, et al. 2017). The third factor that determines the success of paleogenomic approaches is the variable degree with which environmental DNA penetrates into the bone during burial. This environmental DNA dilutes the endogenous DNA and can render shotgun sequencing very costly.

The best bones for the paleogenomic approach are those that contain on one hand sufficient endogenous DNA and on the other hand low amounts of environmental DNA. Several parameters are prone to produce variability with respect to these two properties of ancient bones. First, the initial DNA degradation is susceptible to vary according to the degree of accessibility of the interior of the bone to putrefying microorganisms. The more the bone is vascularized and porous, the easier it is for microorganisms to penetrate into it. The bone parts that contained *in vivo* the highest amounts of dead osteocytes, i.e., bone cells that are embedded in the mineral matrix that they produce, that can escape phagocytosis by osteoclasts during bone remodeling, are the ones with the highest likelihood of containing preserved DNA. This is the case for the petrosal that undergoes very little remodeling after childhood (Sørensen and Bretlau 1997), which may be one of the reasons explaining its high endogenous DNA content. Other *in vivo* calcification processes, such as those transforming dental plaques in calculi, lead to excellent DNA preservation, too (Warinner, et al. 2015). Second, long term degradation is mainly ruled by physicochemical factors (temperature, hygrometry, pH, etc.) that act upon the bone during the entire period spanning the moment of death of the organism to the moment of excavation of the skeletal part. These factors influence the kinetics of the decay reactions and are probably responsible for the large variability between burial sites with the result that even ancient petrous bones can lack any endogenous DNA. With the exception of the thermal age (Smith, et al.

2001) and empirical observations (e.g., (Pruvost, et al. 2007; Pruvost, et al. 2008)), we do not have clues allowing us to identify the archeological or paleontological sites that are favorable for DNA preservation. Finally, the compactness of bones may also favor exclusion of environmental DNA.

The outermost layer of long bones is a new potential source for paleogenomic results that calls for great attention with respect to its exposure to contamination with DNA from excavators and other researchers manipulating the bone prior to the paleogenomic analysis. If this contamination can be minimized through particular precautions during the excavation, the approach developed by the group of M. Meyer based on the treatment with bleach of the bone powder should be taken into consideration (Korlevic, et al. 2015). This approach is susceptible to degrade preferentially exogenous and recent environmental DNA that is not associated intimately with the mineral matrix of the bone, despite variability in the loss of exogenous but also endogenous DNA. The combination of the approaches proposed by Alberti et al. (2018) and Korlević et al. (2015) could allow the recovery of paleogenomic information in cases where petrous bones are not available for analysis. Such a procedure could crack the secret of many more ancient bones.

Figure1: CT scans of a petrous and a long bone showing typical locations of the areas that were sampled for paleogenomic analyses by Alberti et al (2018). The boxplots represent the distribution of the percentage of endogenous DNA in the various sample types reported in Table 1 of Alberti et al. (2018) enriched with the data from petrous bone of Pinhasi et al (2015), using the A and C areas as surrogates of the trabecular and otic capsule regions, respectively. The numbers indicate the number of samples analyzed.

AUTHOR CONTRIBUTIONS

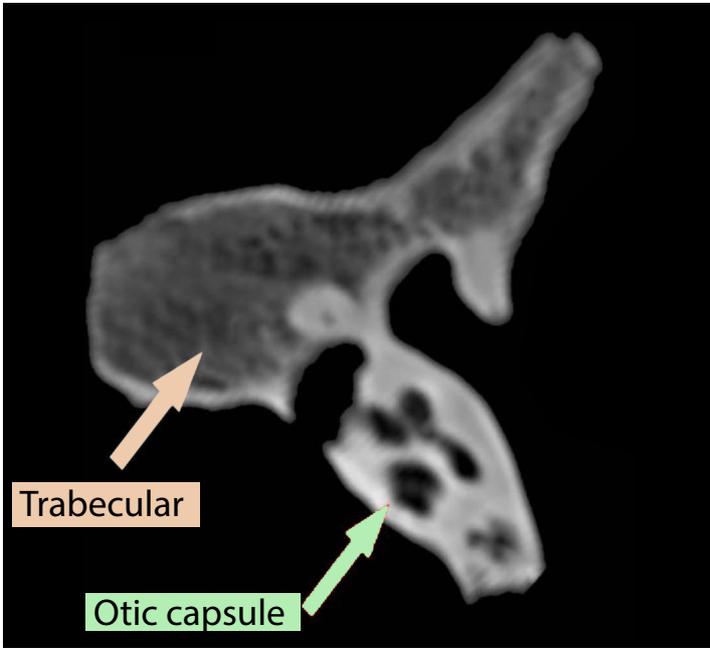
Both EMG and TG conceived and wrote the manuscript.

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Petrous bone



Long bone

