学位論文 （要約）

Application of new analytical method in biological archaeology: proteomics of archaeological human bones and DNA analysis of dental calculus

(生物考古学における新たな分析手法の応用:
古人骨プロテオミクス解析と歯石 DNA 分析)

平成 28 年 12 月博士 (理学) 申請 東京大学大学院理学系研究科
生物科学専攻 澤藤りかい

Application of new analytical method in biological archaeology: proteomics of archaeological human bones and DNA analysis of dental calculus

Rikai Sawafuji

A dissertation submitted to
Graduate School of Science
The University of Tokyo
for the degree
Doctor of Philosophy

December, 2016

Abstract

Over the last four decades, biological archaeology (bioarchaeology) has achieved remarkable growth. Some advances have emerged from improvements in methods and new technologies. On the other hand, problems that can not be clarified by the limitation of conventional bioarchaeological methods still remain. By acquiring information that could not be obtained by conventional methods, it is possible to investigate the past human life from a new viewpoint. The purpose of this study is to apply two new methods to bioarchaeological research.

The first is shotgun proteomics analysis of ancient human bones. Ancient protein analysis provides clues to human life and diseases of ancient times. While immunological methods have been used, the reliability of these methods remains uncertain and cannot be simultaneously applied to multiple proteins. I performed shotgun proteomics of human remains using eight rib bones excavated from the Hitotsubashi site of the Edo period, expecting to acquire physiological information. The output data obtained were analyzed using Gene Ontology and label-free quantification. As a result, a total of 668 proteins were identified, and not only collagen but also various kinds of proteins such as extracellular matrix were identified. I detected leukocyte-derived proteins, possibly originating from the bone marrow of the rib. Particularly prevalent and relatively high expression of eosinophil peroxidase suggests the influence of infectious diseases at that time. This scenario is plausible, considering the overcrowding

and unhygienic living conditions of the Edo city described in the historical literature. I also observed age-dependent differences in proteome profiles, particularly for proteins involved in developmental processes. Among them, alpha-2-HS-glycoprotein (AHSG) demonstrated a strong negative correlation with age. There is a possibility that age information is estimated using the expression level of proteins correlated with age such as AHSG.

The second is food DNA analysis from dental calculus. Isotopic analysis and starch grain analysis have been used as methods of reconstructing ancient diet. The limitation of these methods is the difficulty of species/genus identification. Moreover, organs such as leaves, stems, and roots are soft and hard to remain at a site, which prevents the reconstruction of diet inclusively. In order to investigate diet diversity of the past, calculus samples of human remains excavated from the Unko-in site in the Edo period were collected. PCR amplification and sequencing were performed using the genus *Oryza* specific primer set, because rice (*Oryza sativa*) is the staple food and the only genus present in Japan at that time. The sequence was detected from more than half of the individuals, and this result indicates that ancient calculus contains food DNA. Furthermore, in order to know dietary diversity, DNA metabarcoding targeting plants, animals (meat and fish) and fungi was carried out using eight individuals which showed the genus *Oryza* DNA amplification. As a result, seven families and ten genera of plants were detected in total. Most of the taxonomic groups are present in Japan, but the only one that does not exist is the family *Dipterocarpaceae* inhabiting the tropical lowlands.

At that time, borneol (*ryunou* in Japanese) derived from *Dryobalanops aromatica* was used as a component of tooth powder, so the taxon is likely to be derived from the debris of tooth powder. Some fungi were also detected from the calculus, despite animals except for human were not detected. From these results it is suggested that analysis of dental calculus provides information about food diversity and lifestyle habits of the past.

These methods offer new insights into bioarchaeology and complement conventional methods to reconstruct human life of the past more comprehensively.

Contents

1 General Introduction

1.1 Methods of biological archaeology

Biological archaeology (bioarchaeology) is the scientific study of biological remains from archaeological sites. This field began just four decades ago (Buikstra, 1977), by combining elements of physical anthropology and archaeology (Klaus, 2014; Stojanowski and Duncan, 2015). Now bioarchaeology relies on interdisciplinary research. It is related to anthropology, archaeology, ethnology, history, geography, geology, physics, chemistry, paleoecology, paleontology, paleozoology and paleobotany. Thus, the field has incorporated methods of other fields and evolved. For example, stable isotope analysis has been used extensively in the field of geology and ecology, and it applied to archaeological bones for investigating past diet since the 1970–1980s (Katzenberg, 2008). As the field matures, associated fields have developed such as reconstruction of breastfeeding and weaning practices (Tsutaya and Yoneda, 2015). The emergence of next generation sequencing (NGS) technologies has also enabled ancient DNA studies to evolving into a powerful research tool. This field has entered the new era of genomics and has provided valuable information about past human history (Der Sarkissian et al., 2015). It has enabled the analysis of the genomes of Neanderthals, Denisovan, and Middle Pleistocene hominoids (Meyer et al., 2016, 2012; Prüfer et al., 2014). In recent years, it has also accelerated ancient population analysis, dealing with more than one hundred individuals (Haak et al., 2015; Lazaridis et al., 2016). These results provided

information of the evolutionary and the migration history of humans in detail.

1.1.1 Proteomic Research

In recent years, not only paleogenomics but also paleoproteomics attracts much attention. Lots of questions that analyses of ancient DNA cannot answer have left behind so far, which makes it difficult to obtain the full picture regarding human life of the past. Now thanks to remarkable improvement of the mass spectrometry (MS) and protein databases, ancient protein has been gradually giving the clue of these questions. For example, proteins are more prone to be preserved than DNA (Wang et al., 2012), so they could be extended further back in time. Although the time depth of ancient DNA analyses is ∼400,000 years in temperate areas and ∼700,000 years in permafrost (Dabney et al., 2013a; Meyer et al., 2014; Orlando et al., 2013), ancient proteins from bones sometimes remain over one hundred million years (Schweitzer et al., 2014). Species identification and phylogenetic relationships based on the protein analysis are sometimes more preferable than that by DNA analysis due to this reason (Buckley and Wadsworth, 2014; Welker et al., 2015). Furthermore, protein expression is specific to different tissues, developmental phases, disease or biological processes (Cappellini et al., 2014a). Taking advantage of the features, several studies revealed human diseases of the past. Shotgun proteomics applied to human medieval dental calculus identified bacterial virulence factors and host immune defense proteins (Warinner et al., 2014b). Mummified remains are well-used samples for detecting a host immune system response

to severe bacterial infection (Corthals et al., 2012; Hendy et al., 2016; Maixner et al., 2016). Detecting disease-associated proteins from human bone is promising for contributing to paleopathology, too (Bona et al., 2014; Boros-Major et al., 2011; Kendall et al., 2016).

1.1.2 Ancient plant DNA

The analyses of past diet are often difficult. Especially the consumption of plant foods is difficult to analyze because plants are hardly remained at a site. Most plant materials are subject to rapid decomposition and thus the majority of archaeological plant materials is preserved in a charred state (Nistelberger et al., 2016). This prohibits the reconstruction of diet comprehensively. For example, Habu (2015) discussed the mechanisms of long-term changes in ecosystems and subsistence-settlement systems (economic and social systems) from the perspective of historical ecology and resilience theory. Because of the difficulty of evaluating food diversity directly, the author used lithic assemblage diversity as a substitute for food and subsistence diversity. Thus, food diversity is the capital information for reconstruction of human behavior of the past although the method of analysis is undeveloped.

1.1.3 Objectives of this study

In this study, I focused on applying new methods to bioarchaeology for overcoming these challenges and shedding light on the critical piece of human behavior. In chapter

2, I applied shotgun proteomics analysis for ancient human bones for the first time. The analysis revealed that levels of some proteins existed in bones are correlated with age at death of individuals and that traces of past immune responses remained in bones. In chapter 3, I applied DNA metabarcoding analysis of plants for ancient human calculi for the first time. The analysis demonstrated that the information of ancient food taxa at the genus levels could be extracted from calculus samples and also revealed a past human habit such as brushing teeth. In chapter 4, I explained the achievement, comparison with conventional methods, limitations, and prospective of these two methods.

2 Proteomic profiling of archaeological human bone

本章については、Royal Society Open Science で公表前のため、非公開。

3 Ancient calculus DNA analysis of foods

本章については、5 年以内に雑誌等で刊行予定のため、非公開。

4 General Discussion

4.1 Proteomic profiling of archaeological human bone

4.1.1 Advantages

Proteomic analysis has four advantages when compared with DNA analysis.

a) Longer preservation. Ancient DNA can be analyzed up to ∼700,000 years old at most, in case of permafrost (Orlando et al., 2013). Ancient DNA is susceptible to fragmentation and chemical modification, and the state of preservation of the sample depends greatly on the place that the sample excavated and stored. It is well preserved in a dry and cold climate like Europe, and this allows determination of Neanderthal genomes (Noonan et al., 2006). Meanwhile it is extremely difficult to analyze ancient DNA of remains in acidic soil environment with humidity like in Japan, even if the sample is only thousands of years old.

However, it has been demonstrated in several studies that it will be traced back to at least over a million years ago in regard to ancient protein (Buckley and Wadsworth, 2014; Demarchi et al., 2016; Schweitzer et al., 2014). There is a case that ancient DNA could not be analyzed but ancient protein could be analyzed from the same sample (Welker et al., 2015). These suggests that ancient proteins tend to preserve in bones longer than ancient DNA. Several reasons for longer preservation of ancient proteins are proposed in previous studies. In regard to collagen, a straightforward explanation

is rich content (Collins et al., 2002) and limited solubility (Perumal et al., 2008; Trueman and Martill, 2002), and both provide excellent prerequisites for the survival of the type I collagen. Wang et al. (2012) reported that the elevated abundance of thermostable amino acid residues in type I collagens contributes to its survival. Demarchi et al. (2016) proposed other hypothesis that peptides which has the domain with the strongest calculated binding energy to the mineral surface is selectively preserved in bone.

The reason that I expected to be one of the cause of ancient protein superiority is that ancient protein identification is easier than ancient DNA alignment, owing to the variety of amino acid type. In other words, proteins can be identified by shorter sequence than DNA because of the rich variety of units that make up the sequence. Average molecular weight of an amino acid is 110 daltons, and that of single-strand DNA base is 330 daltons. More than five peptides are commonly used as the minimum peptide length for identifying proteins (Kim et al., 2014; Marcus, 2012), which is too short for DNA to be identified its species and regions. There are 6.4×10^7 types for six peptides ideally, which corresponds to 12 bp. Determination of gene or taxon from 12 bp fragment of DNA is absolutely impossible. This is a simple estimation and energy calculation of organic chemistry is needed, but this character may be one of the reason for easy identification of proteins from older samples.

b) Minimum sample requirement. Molecular biology experiments are basically crushing experiments. Therefore, it is an advantage to be able to analyze with as

small samples as possible. When using bones or teeth as samples, approximately 200 mg to 3 g is necessary for DNA analysis. On the other hand, in the case of proteins, samples can be analyzed if they are approximately ∼40 mg. This enables the protein analysis of even only a small amount of deposits of pottery (Solazzo et al., 2008).

c) Lower contamination. Contamination of modern DNA is a serious problem when analyzing ancient DNA, and it is necessary to use special equipment which prevent contamination from the outside. On the other hand, in the case of ancient proteins, contamination is unlikely to strongly impact on the analysis compared with DNA. This seems to be mainly because of three reasons; (1) more residual amount, (2) no amplification process like PCR (This process can skew the original abundance ratio), and (3) tissue-specific expression pattern which makes it easy to discriminate contaminating proteins (e.g., keratin of skin).

d) Information not available in DNA. Basically, genome is identical in every somatic cell in our bodies. However, the expression pattern of proteins varies between tissues and cells. For example, when analyzing dental calculus, it is impossible to distinguish between beef and milk by DNA analysis, albeit possible by protein analysis. Thus, ancient protein analysis is quite effective in investigating human activities such as the origins of dairy farming (Warinner et al., 2014a).

4.1.2 Limitations

As a disadvantage of ancient protein analysis, there are few differences of sequences between species and among individuals, due to its codon degeneracy. This means that protein analysis is not appropriate for species identification or screening genetic information for each individual. There is also a possibility that the results of protein analysis may be affected by the extraction method (Jiang et al., 2007). Considerable caution is needed when comparing samples extracted by different methods.

4.1.3 Perspectives

It is conceivable that physiological information of individuals such as pregnancy and disease could be obtained from analyzing ancient bone proteins. In this study, it was revealed that various kinds of blood proteins were present in ancient bones. This indicates that not only proteins expressed in bone but also proteins expressed in blood can be detected from ancient bones.

Pregnancy-associated plasma protein-A (PAPP-A) is one of proteins of placental origin found in high concentrations in the blood of pregnant women (Boldt and Conover, 2007). Human placental lactogen (hPL) and human chorionic gonadotropin (hCG) also have essential roles in pregnancy and expressed in the blood. Detecting these proteins from ancient bone will lead to the pregnancy status of past human populations.

As for disease, Schultz et al. (2007) reported that they detected PSA (prostate specific antigen), which is an important marker for the diagnosis of prostate cancer. Cancer of the prostate gland, lung, breast, kidney, stomach, thyroid gland as well as multiple myeloma frequently metastasize to bone. In the case of those diseases which metastasize to bone and diseases which change the state of blood, there is a possibility of revealing the disease by analyzing proteins within bone.

4.2 Ancient calculus DNA analysis of foods

4.2.1 Advantages

The advantage of food analysis of calculus is genus/species level identification. Species-level identification was not tested in this study, but it is possible in theory. This method also enables analysis of organs hardly remained at the site (e.g., leaves, roots, and rhizomes). This method is complement to other methods such as isotope and starch analyses.

In regard to the merit of metabarcoding, it is more cost-effective and suit for population analysis. It is also compatible with screening food DNA at that time. If an interesting taxon is detected by this method, one can expand more specific research, for example, examining nuclear genes or correlation with oral microbiota.

4.2.2 Limitations

The most important problem is contamination with modern DNA, especially soil DNA. There is a possibility that some ancient calculus DNA was contaminated by soil. It is difficult to exclude totally the effect of soil DNA, but there are some solutions for decreasing it.

a) Excluding soil from calculus surface as much as possible. Sometimes the surface of calculus was covered with soil. This should be excluded thoroughly by brushing, scraping, or washing. As for ancient bones and teeth, the outer surface of the samples was often removed using a dental drill (Adachi et al., 2009), but applying this step to the calculus samples is not so easy. The use of only inner calculus (the adjacent part to the teeth) may be desirable, but calculus tends to be very thin, small and brittle. So, UV irradiation of the surface is more practical step for calculus analysis. Some previous studies used EDTA as a decontamination step (Ozga et al., 2016; Warinner et al., 2014b). Others even used bleach for decontamination (Warinner et al., 2014b; Weyrich et al., 2017). However, artificial depurination can be caused by bleach treatment used for decontamination (García-Garcerà et al., 2011). Depurination is a key component of post-mortem fragmentation of ancient DNA molecules (Dabney et al., 2013b; Hofreiter et al., 2001), so one must pay serious attention to it when using bleach for decontamination, if there is need for checking ancient DNA authenticity (Kihana et al., 2013).

b) Comparing with soil as much as possible. Soil sampling from mandibular foramen was not so much, about 20 mg from each foramen. It is good if one can participate in excavation for sampling a large amount of soil. Improving DNA extraction methods from soil is also desirable, as impure substances in soil may inhibit enzyme reaction. In the field of ecology, Extraction kits of MO BIO Laboratories, Inc. are often used for soil samples (Epp et al., 2012; Young et al., 2014).

c) Population analysis. Population analysis is also pivotal for the confidence of identified taxon. The more samples one uses, the more reliable the results are. Ideally, it is better to show that there is a significant difference in regard to certain taxon by comparing the calculus samples and the soil samples.

d) Combination with other methodology. Combining with other methodology such as starch grain analysis and proteomic analysis increases the confidence of existence of identified taxon. This may be efficient especially when analyzing staple foods. Performing PCR with species-specific primer will also efficient for checking whether the identified plant DNA is come from food species or weed.

In the field of starch analysis of calculus, the cases of screening both calculus and soil samples are really rare. Starches in sediments are not analyzed in most starch analysis of calculus (Henry and Piperno, 2008; Piperno and Dillehay, 2008; Wesolowski et al., 2010). This is a serious matter because starch granules basically remain in soil environment (Shibutani et al., 2015). Shibutani (2015) pointed out that it is necessary to collect and analyze the surrounding soil when analyzing starches from

stone tools for checking contamination, although no one depicts the risk of starch analysis from calculus samples without soil samples. As for ancient plant DNA analysis, it is important to examine the sample of soil too.

The other point of limitations is that this method is difficult to estimate the quantity of the food consumption. From the results of starch grain analysis and isotopic analysis, it has been pointed out that calculus may not reflect much of the intake of food. In the starch grain analysis, it was investigated the relationship between plant microremains (the number of starches and phytoliths) in calculus and plant consumption of modern hunter gatherers and chimpanzee populations. They found that starches and phytoliths do record diet, but that the relationship between diet and microremains is not as straightforward (Leonard et al., 2015; Power et al., 2015). According to a study comparing isotopic analysis of bone and calculus, the first attempt is to investigate the potential of calculus as a substitution of bone for reconstructing diet (Scott and Poulson, 2012). However it was revealed that the carbon and nitrogen isotope values of bone and calculus do not correlate, or have a weak correlation (Eerkens et al., 2014; Salazar-García et al., 2014). From these results, it is better to assume that the food residue in the dental calculus preserved by chance rather than cumulatively.

4.2.3 Perspectives

By using this method, diversity of food in the times without literature can be investigated. For example, it is an important issue that whether people during the Jomon

period consumed yams such as *Dioscorea japonica* and *Dioscorea polystachya* commonly. The spread of rice in the Yayoi period and the possibility of eating millet are also issues that ancient calculus could reveal.

It is also a good point of this method to be able to investigate the existence of trade. In this study, the possibility of trade was presented from plants of the family *Dipterocarpaceae*. For this purpose, it is necessary to eliminate and decrease contamination of modern DNA as much as possible to increase certainty.

It is also interesting to analyze the habits of the oral hygiene. Although I discussed the use of tooth powder this time, in the previous study the use of medicinal herbs has been discussed in starch grain, chemical analysis of calculus (Buckley et al., 2014; Hardy et al., 2012). Combining multiple methods for calculus analysis lead to unveiling the past human life more deeply and certainly.

There is room for improvement of the method. Analyzing with DNA hybridization capture instead of with PCR for DNA metabarcoding enables to reduce the effect of contamination and to detect deamination which is the authenticity of ancient DNA. It also allows the analysis of nuclear genes involved in domestication such as shattering resistance. Nistelberger et al. (2016) reported that high-throughput sequencing technologies are not suitable for use with charred archaeobotanicals. There is a possibility that the genome can be analyzed from the food debris of calculus which is not charred.

Funding

This study was supported by Ministry of Education, Culture, Sports, Science, and Technology in Japan, Advanced Graduate Program from the Japan Society for the Promotion of Science, and a grants-in-aid of The Manabu Yoshida Memorial Fund for Scientific Studies on Cultural Properties, Tokyo, Japan.

Acknowledgments

I would like to thank my supervisor, Shintaroh Ueda for all help and encouragement for research and writing manuscripts. My collaborator, Enrico Cappellini, Tomohito Nagaoka, Anna Katerina Fotakis, Rosa Rakownikow Jersie-Christensen, Jesper V. Olsen, Kazuaki Hirata and Aiko Saso gave many supports and advice to my research. I also thank Fuzuki Mizuno, Masahiko Kumagai and the members of Laboratory for Molecular Anthropology and Molecular Evolution of the University of Tokyo for discussion and assistance of my research. I also thank Hirokazu Tsukaya for helpful comment to taxa and usage of plants. Takumi Tsutaya gave me insightful comments to my research and the manuscripts. I also thank my family for all kindness and help.

5 References

Acharya, K. R. and Ackerman, S. J. (2014). Eosinophil granule proteins: Form and function. *J. Biol. Chem.*, 289(25):17406–17415.

Adachi, N., Shinoda, K. I., Umetsu, K., and Matsumura, H. (2009). Mitochondrial DNA analysis of jomon skeletons from the funadomari site, hokkaido, and Its implication for the origins of native american. *Am. J. Phys. Anthropol.*, 138(3):255–265.

Adler, C. J., Dobney, K., Weyrich, L. S., Kaidonis, J., Walker, A. W., Haak, W., Bradshaw, C. J. a., Townsend, G., Sołtysiak, A., Alt, K. W., Parkhill, J., and Cooper, A. (2013). Sequencing ancient calcified dental plaque shows changes in oral microbiota with dietary shifts of the Neolithic and Industrial revolutions. *Nat. Genet.*, 45(4):450–455.

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. (1990). Basic local alignment search tool. *J. Mol. Biol.*, 215(3):403–410.

Alves, R. D. A. M., Demmers, J. A. A., Bezstarosti, K., Van Der Eerden, B. C. J., Verhaar, J. A. N., Eijken, M., and Van Leeuwen, J. P. T. M. (2011). Unraveling the human bone microenvironment beyond the classical extracellular matrix proteins: A human bone protein library. *J. Proteome Res.*, 10(10):4725–4733.

Amulic, B., Cazalet, C., Hayes, G. L., Metzler, K. D., and Zychlinsky, A. (2012). Neutrophil Function: From Mechanisms to Disease. *Annu. Rev. Immunol.*, 30:459–489.

Ascenzi, A., Brunori, M., Citro, G., and Zito, R. (1985). Immunological detection of hemoglobin in bones of ancient Roman times and of Iron and Eneolithic Ages. *Proc. Natl. Acad. Sci.*, 82(November):7170–7172.

Binkertt, C., Demetriou, M., Sukhu, B., Szweras, M., Tenenbaum, H. C., and Dennis, J. W. (1999). Regulation of osteogenesis by fetuin. *J. Biol. Chem.*, 274(40):28514–28520.

Blumenschine, R. J., Peters, C. R., Masao, F. T., Clarke, R. J., Deino, A. L., Hay, R. L., Swiser, C. C., Stanisteert G,, I., Ashley, G. M., Mc Henry J,, L., Sikes, N. E., Merwe, N. J., Tactikos, J. C., Cushing, A. M., Deocampo, D., Njau, J. K., and Ebert, J. I. (2003). Late Pliocene Homo and Hominid Land Usefrom Western Olduvai George, Tanzania. *Science*, 299(5610):1217–1221.

Boldt, H. B. and Conover, C. A. (2007). Pregnancy-associated plasma protein-A (PAPP-A): A local regulator of IGF bioavailability through cleavage of IGFBPs. *Growth Horm. IGF Res.*, 17(1):10–18.

Bona, A., Papai, Z., Maasz, G., Toth, G. A., Jambor, E., Schmidt, J., Toth, C., Farkas, C., and Mark, L. (2014). Mass spectrometric identification of ancient proteins as potential molecular biomarkers for a 2000-year-old osteogenic sarcoma. *PLoS ONE*, 9(1):e87215.

Boros-Major, A., Bona, A., Lovasz, G., Molnar, E., Marcsik, A., Palfi, G., and Mark, L. (2011). New perspectives in biomolecular paleopathology of ancient tuberculosis: A proteomic approach. *J. Archaeol. Sci.*, 38(1):197–201.

Boyer, F., Mercier, C., Bonin, A., Le Bras, Y., Taberlet, P., and Coissac, E. (2016). obitools: A unix-inspired software package for DNA metabarcoding. *Mol. Ecol. Resour.*, 16(1):176–182.

Brandt, L. Ø., Schmidt, A. L., Mannering, U., Sarret, M., Kelstrup, C. D., Olsen, J. V., and Cappellini, E. (2014). Species identification of archaeological skin objects from danish bogs: Comparison between mass spectrometry-based peptide sequencing and microscopy-based methods. *PLoS ONE*, 9(9):e106875.

Brown, K. A. and Brown, T. A. (2013). Biomolecular Archaeology. *Annu. Rev. Anthropol.*, 42(1):159–174.

Bruzek, J. (2002). A method for visual determination of sex, using the human hip bone. *Am. J. Phys. Anthropol.*, 117(2):157–168.

Brylka, L. and Jahnen-Dechent, W. (2013). The role of fetuin-A in physiological and pathological mineralization. *Calcif. Tissue Int.*, 93(4):355–364.

Buckberry, J. and Chamberlain, A. (2002). Age estimation from the auricular surface of the ilium: A revised method. *Am. J. Phys. Anthropol.*, 119(3):231–239.

Buckley, M. and Wadsworth, C. (2014). Proteome degradation in ancient bone: Diagenesis and phylogenetic potential. *Palaeogeogr. Palaeoclimatol. Palaeoecol.*, 416:69–79.

Buckley, S., Usai, D., Jakob, T., Radini, A., and Hardy, K. (2014). Dental calculus reveals unique insights into food items, cooking and plant processing in prehistoric central Sudan. *PLoS ONE*, 9(7):e100808.

Buikstra, J. E. (1977). Biocultural Dimensions of Archaeological Study: A Regional Perspective. In *Biocultural Adapt. Prehist. Am.*, pages 67–84. University of Georgia Press.

Cappellini, E., Collins, M. J., and Gilbert, M. T. P. (2014a). Unlocking Ancient Protein Palimpsests. *Science*, 343(6177):1320–1322.

Cappellini, E., Gentry, A., Palkopoulou, E., Ishida, Y., Cram, D., Roos, A. M., Watson, M., Johansson, U. S., Fernholm, B., Agnelli, P., Barbagli, F., Littlewood, D. T. J., Kelstrup, C. D., Olsen, J. V., Lister, A. M., Roca, A. L., Dalén, L., and Gilbert, M. T. P. (2014b). Resolution of the type material of the Asian elephant, Elephas maximus Linnaeus, 1758 (Proboscidea, Elephantidae). *Zool. J. Linn. Soc.*, 170(1):222–232.

Cappellini, E., Jensen, L. J., Szklarczyk, D., Ginolhac, A., Da Fonseca, R. A. R., Stafford, T. W., Holen, S. R., Collins, M. J., Orlando, L., Willerslev, E., Gilbert, M. T. P., and Olsen, J. V. (2012). Proteomic analysis of a pleistocene mammoth femur reveals more than one hundred ancient bone proteins. *J. Proteome Res.*, 11(2):917–926.

Carpenter, M. L., Buenrostro, J. D., Valdiosera, C., Schroeder, H., Allentoft, M. E., Sikora, M., Rasmussen, M., Gravel, S., Guillén, S., Nekhrizov, G., Leshtakov, K., Dimitrova, D., Theodossiev, N., Pettener, D., Luiselli, D., Sandoval, K., Moreno-Estrada, A., Li, Y., Wang, J., Gilbert, M. T. P., Willerslev, E., Greenleaf, W. J., and Bustamante, C. D. (2013). Pulling out the 1%: Whole-Genome capture for the targeted enrichment of ancient dna sequencing libraries. *Am. J. Hum. Genet.*, 93(5):852–864.

Cattaneo, C., Gelsthorpe, K., Phillips, P., and Sokol, R. J. (1992). Detection of blood proteins in ancient human bone using ELISA: A comparative study of the survival of IgG and albumin. *Int. J. Osteoarchaeol.*, 2(2):103–107.

Cattaneo, C., Gelsthorpe, K., Sokol, R. J., and Phillips, P. (1994). Immunological Detection of Albumin in Ancient Human Cremations using ELISA and Monoclonal Antibodies. *J. Archaeol. Sci.*, 21(4):565–571.

Cecchini, M. G., Hofstetter, W., Halasy, J., Wetterwald, A., and Felix, R. (1997). Role of CSF-1 in bone and bone marrow development. *Mol. Reprod. Dev.*, 46(1):75–84.

Collins, M. J., Nielsen-Marsh, C. M., Hiller, J., Smith, C. I., Roberts, J. P., Prigodich, R. V., Wess, T. J., Csapo, J., Millard, A. R., and Turner-Walker, G. (2002). The survival of organic matter in bone: a review. *Archaeometry*, 44(3):383–394.

Corthals, A., Koller, A., Martin, D. W., Rieger, R., Chen, E. I., Bernaski, M., Recagno, G., and Dávalos, L. M. (2012). Detecting the immune system response of a 500 year-old Inca mummy. *PLoS ONE*, 7(7):e41244.

Cox, J. and Mann, M. (2008). MaxQuant enables high peptide identification rates, individualized p.p.b.-range mass accuracies and proteome-wide protein quantification. *Nat. Biotechnol.*, 26(12):1367–1372.

Cox, J., Neuhauser, N., Michalski, A., Scheltema, R. A., Olsen, J. V., and Mann, M. (2011). Andromeda: A peptide search engine integrated into the MaxQuant environment. *J. Proteome Res.*, 10(4):1794–1805.

Cristy, M. (1981). Active bone marrow distribution as a function of age in humans. *Phys. Med. Biol.*, 26(3):389–400.

Dabney, J., Knapp, M., Glocke, I., Gansauge, M.-T., Weihmann, A., Nickel, B., Valdiosera, C., García, N., Pääbo, S., Arsuaga, J.-L., and Meyer, M. (2013a). Complete mitochondrial genome sequence of a Middle Pleistocene cave bear reconstructed from ultrashort DNA fragments. *Proc. Natl. Acad. Sci. U.S.A.*, 110(39):15758–15763.

Dabney, J., Meyer, M., and Pääbo, S. (2013b). Ancient DNA damage. *Cold Spring Harb. Perspect. Biol.*, 5(7):a012567.

Dallongeville, S., Garnier, N., Rolando, C., and Tokarski, C. (2016). Proteins in Art, Archaeology, and Paleontology: From Detection to Identification. *Chem. Rev.*, 116(1):2–79.

Darbyshire, B. and Henry, R. J. (1981). Differences in fructan content and synthesis in some Allium species. *New Phytol.*, 87(2):249–256.

Dayanandan, S., Ashton, P. S., Williams, S. M., and Primack, R. B. (1999). Phylogeny of the tropical tree family Dipterocarpaceae based on nucleotide sequences of the chloroplast rbcL gene. *Am. J. Bot.*, 86(8):1182–1190.

De Barba, M., Miquel, C., Boyer, F., Mercier, C., Rioux, D., Coissac, E., and Taberlet, P. (2014). DNA metabarcoding multiplexing and validation of data accuracy for diet assessment: Application to omnivorous diet. *Mol. Ecol. Resour.*, 14(2):306–323.

De La Fuente, C., Flores, S., and Moraga, M. (2013). Dna from human ancient bacteria: A novel source of genetic evidence from archaeological dental calculus. *Archaeometry*, 55(4):767–778.

Demarchi, B., Hall, S., Roncal-Herrero, T., Freeman, C. L., Woolley, J., Crisp, M. K., Wilson, J., Fotakis, A., Fischer, R., Kessler, B. M., Jersie-Christensen, R. R., Olsen, J. V., Haile, J., Thomas, J., Marean, C. W., Parkington, J., Presslee, S., Lee-Thorp, J., Ditchfield, P., Hamilton, J. F., Ward, M. W., Wang, C. M., Shaw, M. D., Harrison, T., Domínguez-Rodrigo, M., Macphee, R. D. E., Kwekason, A., Ecker, M., Horwitz, L. K., Chazan, M., Kroger, R., Thomas-Oates, J., Harding, J. H., Cappellini, E.,

Penkman, K., and Collins, M. J. (2016). Protein sequences bound to mineral surfaces persist into deep time. *Elife*, 5:e17092.

Der Sarkissian, C., Allentoft, M. E., Ávila-Arcos, M. C., Barnett, R., Campos, P. F., Cappellini, E., Ermini, L., Fernández, R., da Fonseca, R., Ginolhac, A., Hansen, A. J., Jónsson, H., Korneliussen, T., Margaryan, A., Martin, M. D., Moreno-Mayar, J. V., Raghavan, M., Rasmussen, M., Velasco, M. S., Schroeder, H., Schubert, M., Seguin-Orlando, A., Wales, N., Gilbert, M. T. P., Willerslev, E., and Orlando, L. (2015). Ancient genomics. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.*, 370(1660):20130387.

Dickson, I. R. and Bagga, M. K. (1985). Changes with age in the non-collagenous proteins of human bone. *Connect. Tissue Res.*, 14(1):77–85.

Dodo, Y. (1975). Non-Metric Traits in the Japanese Crania of the Edo Period. *Bull. Natl. Sci. Museum. Ser. D, Anthropol.*, 1:41–54.

Downs, E. F. and Lowenstein, J. M. (1995). Identification of archaeological blood protiens: A cautionary note. *J. Archaeol. Sci.*, 22(1):11–16.

Eerkens, J. W., de Voogt, A., Dupras, T. L., Rose, S. C., Bartelink, E. J., and Francigny, V. (2014). Intra- and inter-individual variation in δ13C and δ15N in human dental calculus and comparison to bone collagen and apatite isotopes. *J. Archaeol. Sci.*, 52:64–71.

Ehara, A., Ishikawa, N., and Syoko, H. (2009). *Nihon shokumotsu-shi.* Yoshikawa Kobunkan, Tokyo (in Japanese).

Epp, L. S., Boessenkool, S., Bellemain, E. P., Haile, J., Esposito, A., Riaz, T., Erséus, C., Gusarov, V. I., Edwards, M. E., Johnsen, A., Stenøien, H. K., Hassel, K., Kauserud, H., Yoccoz, N. G., Bråthen, K. A., Willerslev, E., Taberlet, P., Coissac, E., and Brochmann, C. (2012). New environmental metabarcodes for analysing soil DNA: Potential for studying past and present ecosystems. *Mol. Ecol.*, 21(8):1821–1833.

Eshed, V., Gopher, A., and Hershkovitz, I. (2006). Tooth wear and dental pathology at the advent of agriculture: New evidence from the levant. *Am. J. Phys. Anthropol.*, 130(2):145–159.

Ezkurdia, I., Juan, D., Rodriguez, J. M., Frankish, A., Diekhans, M., Harrow, J., Vazquez, J., Valencia, A., and Tress, M. L. (2014). Multiple evidence strands suggest that theremay be as few as 19 000 human protein-coding genes. *Hum. Mol. Genet.*, 23(22):5866–5878.

Faurschou, M. and Borregaard, N. (2003). Neutrophil granules and secretory vesicles in inflammation. *Microbes Infect.*, 5(14):1317–1327.

Fujikawa, Y. (1969). *Nihon-shippei-shi*. Heibonsha, Tokyo (in Japanese).

García-Garcerà, M., Gigli, E., Sanchez-Quinto, F., Ramirez, O., Calafell, F., Civit, S., and Lalueza-Fox, C. (2011). Fragmentation of contaminant and endogenous dna in ancient samples determined by shotgun sequencing; prospects for human palaeogenomics. *PLoS ONE*, 6(8):e24161.

Grealy, A., Douglass, K., Haile, J., Bruwer, C., Gough, C., and Bunce, M. (2016a). Tropical ancient DNA from bulk archaeological fish bone reveals the subsistence practices of a historic coastal community in southwest Madagascar. *J. Archaeol. Sci.*, 75:82–88.

Grealy, A., Macken, A., Allentoft, M. E., Rawlence, N. J., Reed, E., and Bunce, M. (2016b). An assessment of ancient DNA preservation in Holocene-Pleistocene fossil bone excavated from the world heritage Naracoorte Caves, South Australia. *J. Quat. Sci.*, 31(1):33–45.

Haak, W., Lazaridis, I., Patterson, N., Rohland, N., Mallick, S., Llamas, B., Brandt, G., Nordenfelt, S., Harney, E., Stewardson, K., Fu, Q., Mittnik, A., Bánffy, E., Economou, C., Francken, M., Friederich, S., Pena, R. G., Hallgren, F., Khartanovich, V., Khokhlov, A., Kunst, M., Kuznetsov, P., Meller, H., Mochalov, O., Moiseyev, V., Nicklisch, N., Pichler, S. L., Risch, R., Rojo Guerra, M. a., Roth, C., Szécsényi-Nagy, A., Wahl, J., Meyer, M., Krause, J., Brown, D., Anthony, D., Cooper, A., Alt, K. W., and Reich, D. (2015). Massive migration from the steppe was a source for Indo-European languages in Europe. *Nature*, 522(7555):207–211.

Habu, J. (2015). Mechanisms of long-term culture change and human impacts on the environment : A perspective from historical ecology, with special reference to the Early and Middle Jomon periods of prehistoric Japan. *Quat. Res.*, 54(5):299–310.

Hanihara, T., Ishida, H., Ohshima, N., Kondo, O., and Masuda, T. (1994). Dental calculus and other dental disease in a human skeleton of the Okhotsk Culture unearthed at Hamanaka-2 site, Rebun-Island, Hokkaido, Japan. *Int. J. Osteoarchaeol.*, 4(4):343–351.

Hardy, K., Buckley, S., Collins, M. J., Estalrrich, A., Brothwell, D., Copeland, L., García-Tabernero, A., García-Vargas, S., De La Rasilla, M., Lalueza-Fox, C., Huguet, R., Bastir, M., Santamaría, D., Madella, M., Wilson, J., Cortés, Á. F., and Rosas, A. (2012). Neanderthal medics? Evidence for food, cooking, and medicinal plants entrapped in dental calculus. *Naturwissenschaften*, 99(8):617–626.

Hardy, K., Radini, A., Buckley, S., Blasco, R., Copeland, L., Burjachs, F., Girbal, J., Yll, R., Carbonell, E., and María Bermúdez De Castro, J. (2017). Diet and environment 1.2 million years ago revealed through analysis of dental calculus from Europe's oldest hominin at Sima del Elefante, Spain. *Sci. Nat.*, 104(2).

Hebert, P. D. N. and Gregory, T. R. (2005). The promise of DNA barcoding for taxonomy. *Syst. Biol.*, 54(5):852–859.

Hendy, J., Collins, M., Teoh, K. Y., Ashford, D. A., Thomas-Oates, J., Donoghue, H. D., Pap, I., Minnikin, D. E., Spigelman, M., and Buckley, M. (2016). The challenge of identifying tuberculosis proteins in archaeological tissues. *J. Archaeol. Sci.*, 66:146–153.

Henry, A. G. and Piperno, D. R. (2008). Using plant microfossils from dental calculus to recover human diet: a case study from Tell al-Raq'i, Syria. *J. Archaeol. Sci.*, 35(7):1943–1950.

Hershkovitz, I., Kelly, J., Latimer, B., Simpson, S., Polak, J., and Rosenberg, M. (1997). Oral bacteria in Miocene Sivapithecus. *J. Hum. Evol.*, 33:507–512.

Hill, R. C., Wither, M. J., Nemkov, T., Barrett, A., D'Alessandro, A., Dzieciatkowska, M., and Hansen, K. C. (2015). Preserved proteins from extinct bison Latifrons identified by tandem mass spectrometry; hydroxylysine glycosides are a common feature of ancient collagen. *Mol. Cell. Proteomics*, 14(7):1946–1958.

Hirata, K. (1990). Secular trend and age distribution of cribra orbitalia in Japanese. *Hum. Evol.*, 5(4):375–385.

Hofreiter, M., Serre, D., Poinar, H. N., Kuch, M., and Pääbo, S. (2001). Ancient DNA. *Nat. Rev. Genet.*, 2(5):353–359.

Horiuchi, K., Amizuka, N., Takeshita, S., Takamatsu, H., Katsuura, M., Ozawa, H., Toyama, Y., Bonewald, L. F., and Kudo, a. (1999). Identification and characterization of a novel protein, periostin, with restricted expression to periosteum and periodontal ligament and increased expression by transforming growth factor beta. *J. Bone Miner. Res.*, 14(7):1239–1249.

Hozumi, H. (1693). *Kyumin myoyaku*. Ibaraki, T (in Japanese).

Ishihama, Y., Oda, Y., Tabata, T., Sato, T., Nagasu, T., Rappsilber, J., and Mann, M. (2005). Exponentially modified protein abundance index (emPAI) for estimation of absolute protein amount in proteomics by the number of sequenced peptides per protein. *Mol. Cell. Proteomics*, 4(9):1265–1272.

Iwatsuki, K., Boufford, D. E., and Ohba, H. (1995). *Flora of Japan: Pteridophyta and Gymnospermae*. Kodansha, Tokyo.

Jang, Y., Jang, S., Lee, J., Lee, H., Lim, Y. W., Kim, C., and Kim, J.-J. (2016). Diversity of Wood-Inhabiting Polyporoid and Corticioid Fungi in Odaesan National Park, Korea. *Mycobiology*, 44(4):217–236.

Jiang, X., Ye, M., Jiang, X., Liu, G., Feng, S., Cui, L., and Zou, H. (2007). Method development of efficient protein extraction in bone tissue for proteome analysis. *J. Proteome Res.*, 6(6):2287–2294.

Kaifu, Y., Kono, R. T., Sutikna, T., Saptomo, E. W., Jatmiko, Awe, R. D., and Baba, H. (2015). Descriptions of the dental remains of Homo floresiensis. *Anthropol. Sci.*, 123(2):129–145.

Katzenberg, M. A. (2008). Stable isotope analysis: a tool for studying past diet, demography, and life history. In *Biol. Anthropol. Hum. Skelet.*, pages 413–441.

Kendall, R., Hendy, J., Collins, M. J., Millard, A. R., and Gowland, R. L. (2016). Poor preservation of antibodies in archaeological human bone and dentine. *Sci. Technol. Archaeol. Res.*, 2(1):15–24.

Kessels, M. Y., Huitema, L. A. F., Boeren, S., Kranenbarg, S., Schulte-Merker, S., Van Leeuwen, J. L., and De Vries, S. C. (2014). Proteomics analysis of the zebrafish skeletal extracellular matrix. *PLoS ONE*, 9(3):e90568.

Kihana, M., Mizuno, F., Sawafuji, R., Wang, L., and Ueda, S. (2013). Emulsion PCR-coupled target enrichment: An effective fishing method for high-throughput sequencing of poorly preserved ancient DNA. *Gene*, 528(2):347–351.

Kim, M.-S., Pinto, S., Getnet, D., Nirujogi, R., Manda, S., Chaerkady, R., Madugundu, A., Kelkar, D., Isserlin, R., Jain, S., Thomas, J., Muthusamy, B., Pamela, L.-R., Kumar, P., Sahasrabuddhe, N., Balakrishnan, L., Advani, J., George, B., Renuse, S., Selvan, L., Patil, A., Nanjappa, V., Radhakrishnan, A., Prasad, S., Subbannayya, T., Raju, R., Kumar, M., Sreenivasamurthy, S., Marimuthu, A., Sathe, G., Chavan, S., Datta, K., Subbannayya, Y., Sahu, A., Yelamanchi, S., Jayaram, S., Rajagopalan, P., Sharma, J., Murthy, K., Syed, N., Goel, R., Khan, A., Ahmad, S., Dey, G., Mudgal, K., Chatterjee, A., Huang, T.-C., Zhong, J., Wu, X., Shaw, P., Freed, D., Zahari, M., Mukherjee, K., Shankar, S., Mahadevan, A., Lam, H., Mitchell, C., Shankar, S., Satishchandra, P., Schroeder, J., Sirdeshmukh, R., Maitra, A., Leach, S., Drake, C., Halushka, M., Prasad, T., Hruban, R., Kerr, C., Bader, G., Christine, I.-D., Gowda, H., and Pandey, A. (2014). A draft map of the human proteome. *Nature*, 509(7502):575–581.

Kitagawa, M. (1853). *Morisada-manko*.

Klaus, H. D. (2014). Frontiers in the bioarchaeology of stress and disease: Cross-disciplinary perspectives from pathophysiology, human biology, and epidemiology. *Am. J. Phys. Anthropol.*, 155(2):294–308.

Kolman, C. J., Centurion-Lara, A., Lukehart, S. A., Owsley, D. W., and Tuross, N. (1999). Identification of Treponema pallidum subspecies pallidum in a 200-year-old skeletal specimen. *J. Infect. Dis.*, 180(6):2060–2063.

Kruzynska-Frejtag, A., Machnicki, M., Rogers, R., Markwald, R. R., and Conway, S. J. (2001). Periostin (an osteoblast-specific factor) is expressed within the embryonic mouse heart during valve formation. *Mech. Dev.*, 103(1-2):183–188.

Laughlin, G. A., Barrett-Connor, E., Cummins, K. M., Daniels, L. B., Wassel, C. L., and Ix, J. H. (2013). Sex-specific association of fetuin-a with type 2 diabetes in older community-dwelling adults: The Rancho Bernardo study. *Diabetes Care*, 36(7):1994–2000.

Lazaridis, I., Nadel, D., Rollefson, G., Merrett, D. C., Rohland, N., Mallick, S., Fernandes, D., Novak, M., Gamarra, B., Sirak, K., Connell, S., Stewardson, K., Harney, E., Fu, Q., Gonzalez-Fortes, G., Jones, E. R., Roodenberg, S. A., Lengyel, G., Bocquentin, F., Gasparian, B., Monge, J. M., Gregg, M., Eshed, V., Mizrahi, A.-S., Meiklejohn, C., Gerritsen, F., Bejenaru, L., Blüher, M., Campbell, A., Cavalleri, G., Comas, D., Froguel, P., Gilbert, E., Kerr, S. M., Kovacs, P., Krause, J., McGettigan, D., Merrigan, M., Merriwether, D. A., O'Reilly, S., Richards, M. B., Semino, O., Shamoon-Pour, M., Stefanescu, G., Stumvoll, M., Tönjes, A., Torroni, A., Wilson, J. F., Yengo, L., Hovhannisyan, N. A., Patterson, N., Pinhasi, R., and Reich, D. (2016). Genomic insights into the origin of farming in the ancient Near East. *Nature*, 536(7617):1–22.

Leonard, C., Vashro, L., O'Connell, J. F., and Henry, A. G. (2015). Plant microremains in dental calculus as a record of plant consumption: A test with Twe forager-horticulturalists. *J. Archaeol. Sci. Reports*, 2:449–457.

Lozano, M., Subirà, M. E., Aparicio, J., Lorenzo, C., and Gómez-Merino, G. (2013). Toothpicking and Periodontal Disease in a Neanderthal Specimen from Cova Foradà Site (Valencia, Spain). *PLoS ONE*, 8(10):6–11.

Mahon, A. R., Nathan, L. R., and Jerde, C. L. (2014). Meta-genomic surveillance of invasive species in the bait trade. *Conserv. Genet. Resour.*, 6(3):563–567.

Maixner, F., Krause-Kyora, B., Turaev, D., Herbig, A., Hoopmann, M. R., Hallows, J. L., Kusebauch, U., Vigl, E. E., Malfertheiner, P., Megraud, F., O'Sullivan, N.,

Cipollini, G., Coia, V., Samadelli, M., Engstrand, L., Linz, B., Moritz, R. L., Grimm, R., Krause, J., Nebel, A., Moodley, Y., Rattei, T., and Zink, A. (2016). The 5300-year-old Helicobacter pylori genome of the Iceman. *Science*, 351(6269):162–165.

Maki, J., Sakagami, H., Kuwada, M., Caceres, A., Sekiya, H., and Tamai, E. (2009). Infections with gastrointestinal parasitic helminths indigenous to Japan and their treatment historically studied in an attempt to control the diseases in countries where they are still rampant: (1) the Jomon to Edo periods. *Yakushigaku zasshi*, 44(1):18–23.

Makino, T. (2008). *New Makino's illustrated flora of Japan*. Hokuryukan, Tokyo (in Japanese).

Marcus, K., editor (2012). *Quantitative Methods in Proteomics*. Humana Press, New York.

Marini, J. C. and Blissett, A. R. (2013). New genes in bone development: what's new in osteogenesis imperfecta. *J. Clin. Endocrinol. Metab.*, 98(8):3095–3103.

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.journal*, 17(1):10–12.

Marvik, O. J., Isaksen, M. L., Roza, L., and Lindqvist, B. H. (1997). Photoimmunodetection: A nonradioactive labeling and detection method for DNA. *Biotechniques*, 23(5):892–896.

Mattinzoli, D., Rastaldi, M. P., Ikehata, M., Armelloni, S., Pignatari, C., Giardino, L. A., Li, M., Alfieri, C. M., Regalia, A., Riccardi, D., and Messa, P. (2016). FGF23-regulated production of Fetuin-A (AHSG) in osteocytes. *Bone*, 83:35–47.

Meyer, M., Arsuaga, J.-L., de Filippo, C., Nagel, S., Aximu-Petri, A., Nickel, B., Martínez, I., Gracia, A., de Castro, J. M. B., Carbonell, E., Viola, B., Kelso, J., Prüfer, K., and Pääbo, S. (2016). Nuclear DNA sequences from the Middle Pleistocene Sima de los Huesos hominins. *Nature*, 531(7595):504–507.

Meyer, M., Fu, Q., Aximu-Petri, A., Glocke, I., Nickel, B., Arsuaga, J.-L. L., Martinez, I., Gracia, A., de Castro, J. M. B., Carbonell, E., Paabo, S., Martínez, I., Gracia, A., de Castro, J. M. B., Carbonell, E., and Pääbo, S. (2014). A mitochondrial genome sequence of a hominin from Sima de los Huesos. *Nature*, 505(7483):403–406.

Meyer, M., Kircher, M., Gansauge, M.-t., Li, H., Racimo, F., Siebauer, M., Green, R. E., Bryc, K., Briggs, A. W., Stenzel, U., Dabney, J., Shendure, J., Kitzman, J., Slatkin, M., and Reich, D. (2012). A High-Coverage Genome Sequence from an Archaic Denisovan Individual. *Science*, 338(2012):1–14.

Mi, H., Poudel, S., Muruganujan, A., Casagrande, J. T., and Thomas, P. D. (2016). PANTHER version 10: Expanded protein families and functions, and analysis tools. *Nucleic Acids Res.*, 44(D1):D336–D342.

Miya, M., Sato, Y., Fukunaga, T., Sado, T., Poulsen, J. Y., Sato, K., Minamoto, T., Yamamoto, S., Yamanaka, H., Araki, H., Kondoh, M., and Iwasaki, W. (2015). MiFish, a set of universal PCR primers for metabarcoding environmental DNA from fishes: detection of more than 230 subtropical marine species. *R. Soc. Open Sci.*, 2(7):150088.

Miyazaki, Y. (1697). *Nogyo zensyo (in Japanese)*.

Mohan, S., Farley, J. R., and Baylink, D. J. (1995). Age-related changes in IGFBP-4 and IGFBP-5 levels in human serum and bone: implications for bone loss with aging. *Prog. Growth Factor Res.*, 6(2-4):465–473.

Motoi, S. (1794). *Hiden eiseiron (in Japanese)*.

Moulopoulos, L. A. and Koutoulidis, V. (2015). *Bone marrow MRI: A pattern-based approach*. Springer Milan, Milano.

Murray, D. C., Haile, J., Dortch, J., White, N. E., Haouchar, D., Bellgard, M. I., Allcock, R. J., Prideaux, G. J., and Bunce, M. (2013). Scrapheap challenge: a novel bulk-bone metabarcoding method to investigate ancient DNA in faunal assemblages. *Sci. Rep.*, 3:3371.

Nagaoka, T. and Hirata, K. (2007). Reconstruction of paleodemographic characteristics from skeletal age at death distributions: Perspectives from Hitotsubashi, Japan. *Am. J. Phys. Anthropol.*, 134(3):301–311.

Nakagawa, N., Kinosaki, M., Yamaguchi, K., Shima, N., Yasuda, H., Yano, K., Morinaga, T., and Higashio, K. (1998). RANK is the essential signaling receptor for osteoclast differentiation factor in osteoclastogenesis. *Biochem. Biophys. Res. Commun.*, 253(2):395–400.

Nakamura, T., Fox-Robichaud, A., Kikkawa, R., Kashiwagi, A., Kojima, H., Fujimiya, M., and Wong, N. C. (1999). Transcription factors and age-related decline in apolipoprotein A-I expression. *J. Lipid Res.*, 40(9):1709–1718.

Nistelberger, H. M., Smith, O., Wales, N., Star, B., and Boessenkool, S. (2016). The efficacy of high-throughput sequencing and target enrichment on charred archaeobotanical remains. *Sci. Rep.*, 6:37347.

Noonan, J. P., Coop, G., Kudaravalli, S., Smith, D., Krause, J., Alessi, J., Chen, F., Platt, D., Pääbo, S., Pritchard, J. K., and Rubin, E. M. (2006). Sequencing and analysis of Neanderthal genomic DNA. *Science*, 314(5802):1113–1118.

OHTA, T., Hattori, S., Murakami, M., Nishiyama, S., and Matsuda, I. (1989). Age- and sex-related differences in lipoproteins containing apoprotein A-I. *Arterioscler. Thromb. Vasc. Biol.*, 9(1):90–95.

Orlando, L., Ginolhac, A., Zhang, G., Froese, D., Albrechtsen, A., Stiller, M., Schubert, M., Cappellini, E., Petersen, B., Moltke, I., Johnson, P. L. F., Fumagalli, M., Vilstrup, J. T., Raghavan, M., Korneliussen, T., Malaspinas, A.-S., Vogt, J., Szklarczyk, D., Kelstrup, C. D., Vinther, J., Dolocan, A., Stenderup, J., Velazquez, A. M. V., Cahill, J., Rasmussen, M., Wang, X., Min, J., Zazula, G. D., Seguin-Orlando, A., Mortensen, C., Magnussen, K., Thompson, J. F., Weinstock, J., Gregersen, K., Røed, K. H., Eisenmann, V., Rubin, C. J., Miller, D. C., Antczak, D. F., Bertelsen, M. F., Brunak, S., Al-Rasheid, K. A. S., Ryder, O., Andersson, L., Mundy, J., Krogh, A., Gilbert, M. T. P., Kjær, K., Sicheritz-Ponten, T., Jensen, L. J., Olsen, J. V., Hofreiter, M., Nielsen, R., Shapiro, B., Wang, J., and Willerslev, E. (2013). Recalibrating Equus evolution using the genome sequence of an early Middle Pleistocene horse. *Nature*, 499(7456):74–78.

Ozga, A. T., Nieves-Colón, M. A., Honap, T. P., Sankaranarayanan, K., Hofman, C. A., Milner, G. R., Lewis, C. M., Stone, A. C., and Warinner, C. (2016). Successful enrichment and recovery of whole mitochondrial genomes from ancient human dental calculus. *Am. J. Phys. Anthropol.*, 160(2):220–228.

Pedersen, K. O. (1944). Fetuin, a New Globulin Isolated from Serum. *Nature*, 154(3914):575–575.

Perumal, S., Antipova, O., and Orgel, J. P. R. O. (2008). Collagen fibril architecture, domain organization, and triple-helical conformation govern its proteolysis. *Proc. Natl. Acad. Sci. U.S.A.*, 105(8):2824–2829.

Peruzzi, B., Cappariello, A., Del Fattore, A., Rucci, N., De Benedetti, F., and Teti, A. (2012). c-Src and IL-6 inhibit osteoblast differentiation and integrate IGFBP5 signalling. *Nat. Commun.*, 3:630.

Piñol, J., Mir, G., Gomez-Polo, P., and Agustí, N. (2015). Universal and blocking primer mismatches limit the use of high-throughput DNA sequencing for the quantitative metabarcoding of arthropods. *Mol. Ecol. Resour.*, 15(4):819–830.

Piperno, D. R. and Dillehay, T. D. (2008). Starch grains on human teeth reveal early broad crop diet in northern Peru. *Proc. Natl. Acad. Sci. U.S.A.*, 105(50):19622–19627.

Port, J. A., O'Donnell, J. L., Romero-Maraccini, O. C., Leary, P. R., Litvin, S. Y., Nickols, K. J., Yamahara, K. M., and Kelly, R. P. (2016). Assessing vertebrate biodiversity in a kelp forest ecosystem using environmental DNA. *Mol. Ecol.*, 25(2):527–541.

Power, R. C., Salazar-García, D. C., Wittig, R. M., Freiberg, M., and Henry, A. G. (2015). Dental calculus evidence of Taï Forest Chimpanzee plant consumption and life history transitions. *Sci. Rep.*, 5:15161.

Preus, H. R., Marvik, O. J., Selvig, K. A., and Bennike, P. (2011). Ancient bacterial DNA (aDNA) in dental calculus from archaeological human remains. *J. Archaeol. Sci.*, 38(8):1827–1831.

Prüfer, K., Racimo, F., Patterson, N., Jay, F., Sankararaman, S., Sawyer, S., Heinze, A., Renaud, G., Sudmant, P. H., de Filippo, C., Li, H., Mallick, S., Dannemann, M., Fu, Q., Kircher, M., Kuhlwilm, M., Lachmann, M., Meyer, M., Ongyerth, M., Siebauer, M., Theunert, C., Tandon, A., Moorjani, P., Pickrell, J., Mullikin, J. C., Vohr, S. H., Green, R. E., Hellmann, I., Johnson, P. L. F., Blanche, H., Cann, H., Kitzman, J. O., Shendure, J., Eichler, E. E., Lein, E. S., Bakken, T. E., Golovanova, L. V., Doronichev, V. B., Shunkov, M. V., Derevianko, A. P., Viola, B., Slatkin, M., Reich, D., Kelso, J., and Pääbo, S. (2014). The complete genome sequence of a Neanderthal from the Altai Mountains. *Nature*, 505(7481):43–49.

Quelch, K. J., Cole, W. G., and Melick, R. A. (1984). Noncollagenous proteins in normal and pathological human bone. *Calcif. Tissue Int.*, 36(1):545–549.

Riaz, T., Shehzad, W., Viari, A., Pompanon, F., Taberlet, P., and Coissac, E. (2011). EcoPrimers: Inference of new DNA barcode markers from whole genome sequence analysis. *Nucleic Acids Res.*, 39(21):1–11.

Robinson, K. N. and Teran-Garcia, M. (2016). From infancy to aging: Biological and behavioral modifiers of Fetuin-A. *Biochimie*, 124:141–149.

Roza, L., van der Wulp, K. J. M., MacFarlane, S. J., Paul, P. H., and Baan, R. A. (1988). Detection of Cyclobutane Thymine Dimers in Dna of Human Cells With Monoclonal Antibodies Raised Against a Thymine Dimer- Containing Tetranucleotide. *Photochem. Photobiol.*, 48(5):627–633.

Salazar-García, D. C., Richards, M. P., Nehlich, O., and Henry, A. G. (2014). Dental calculus is not equivalent to bone collagen for isotope analysis: A comparison between carbon and nitrogen stable isotope analysis of bulk dental calculus, bone and dentine collagen from same individuals from the Medieval site of El Raval (Alicante. *J. Archaeol. Sci.*, 47(1):70–77.

Schmidt-Schultz, T. H. and Schultz, M. (2004). Bone protects proteins over thousands of years: extraction, analysis, and interpretation of extracellular matrix proteins in archeological skeletal remains. *Am. J. Phys. Anthropol.*, 123(1):30–39.

Schroeter, E. R. and Cleland, T. P. (2016). Glutamine deamidation: An indicator of antiquity, or preservational quality? *Rapid Commun. Mass Spectrom.*, 30(2):251–255.

Schultz, M., Parzinger, H., Posdnjakov, D. V., Chikisheva, T. A., and Schmidt-Schultz, T. H. (2007). Oldest known case of metastasizing prostate carcinoma diagnosed in the skeleton of a 2,700-year-old Scythian King from Arzhan (Siberia, Russia). *Int. J. Cancer*, 121(12):2591–2595.

Schweitzer, M. H., Schroeter, E. R., and Goshe, M. B. (2014). Protein molecular data from ancient (>1 million years old) fossil material: Pitfalls, possibilities and grand challenges. *Anal. Chem.*, 86(14):6731–6740.

Scott, A. B., Choi, K. Y., Mookherjee, N., Hoppa, R. D., and Larcombe, L. A. (2016). The biochemical signatures of stress: A preliminary analysis of osteocalcin concentrations and macroscopic skeletal changes associated with stress in the 13th - 17th centuries black friars population. *Am. J. Phys. Anthropol.*, 159(4):596–606.

Scott, G. R. and Poulson, S. R. (2012). Stable carbon and nitrogen isotopes of human dental calculus: A potentially new non-destructive proxy for paleodietary analysis. *J. Archaeol. Sci.*, 39(5):1388–1393.

Shehzad, W., Riaz, T., Nawaz, M. A., Miquel, C., Poillot, C., Shah, S. A., Pompanon, F., Coissac, E., and Taberlet, P. (2012). Carnivore diet analysis based on next-generation sequencing: Application to the leopard cat (Prionailurus bengalensis) in Pakistan. *Mol. Ecol.*, 21(8):1951–1965.

Shibutani, A. (2015). The Present Issues on Starch Analysis in Japanese Archaeology. *Cult. Antiq.*, 67(1):108–118.

Shibutani, A., Aono, T., and Nagaya, Y. (2015). Examination of Contaminated Materials in Starch Residue Analysis : Focusing on the Kitakogane Shell Mounds in Date City, Hokkaido. *Bull. Natl. Museum Japanese Hist.*, 195:79–110.

Smith, P. R. and Wilson, M. T. (1990). Detection of haemoglobin in human skeletal remains by ELISA. *J. Archaeol. Sci.*, 17(3):255–268.

Solazzo, C., Fitzhugh, W. W., Rolando, C., and Tokarski, C. (2008). Identification of protein remains in archaeological potsherds by proteomics. *Anal. Chem.*, 80(12):4590–4597.

Stojanowski, C. M. and Duncan, W. N. (2015). Engaging bodies in the public imagination: Bioarchaeology as social science, science, and humanities. *Am. J. Hum. Biol.*, 27(1):51–60.

Suzuki, H., Sakura, H., and Akiyoshi, H. (1957). Fukagawa Unko-in shutsudo no Edo jidaijin toukotsu ni tsuite. *Proc. Jt. Meet. Anthropol. Soc. Nippon Japanese Soc. Ethnol. 11th Sess.*, pages 102–105.

Szklarczyk, D., Franceschini, A., Wyder, S., Forslund, K., Heller, D., Huerta-Cepas, J., Simonovic, M., Roth, A., Santos, A., Tsafou, K. P., Kuhn, M., Bork, P., Jensen, L. J., and Von Mering, C. (2015). STRING v10: Protein-protein interaction networks, integrated over the tree of life. *Nucleic Acids Res.*, 43(D1):D447–D452.

Taberlet, P., Coissac, E., Pompanon, F., Gielly, L., Miquel, C., Valentini, A., Vermat, T., Corthier, G., Brochmann, C., and Willerslev, E. (2007). Power and limitations of the chloroplast trnL (UAA) intron for plant DNA barcoding. *Nucleic Acids Res.*, 35(3):e14.

Tedersoo, L., Bahram, M., Polme, S., Koljalg, U., Yorou, N., Wijesundera, R., Ruiz, L., Vasco-Palacios, A., Thu, P., Suija, A., Smith, M., Sharp, C., Saluveer, E., Saitta, A., Rosas, M., Riit, T., Ratkowsky, D., Pritsch, K., Poldmaa, K., Piepenbring, M., Phosri, C., Peterson, M., Parts, K., Partel, K., Otsing, E., Nouhra, E., Njouonkou, A., Nilsson, R., Morgado, L., Mayor, J., May, T., Majuakim, L., Lodge, D., Lee, S., Larsson, K., Kohout, P., Hosaka, K., Hiiesalu, I., Henkel, T., Harend, H., Guo, L., Greslebin, A., Grelet, G., Geml, J., Gates, G., Dunstan, W., Dunk, C., Drenkhan, R., Dearnaley, J., De Kesel, A., Dang, T., Chen, X., Buegger, F., Brearley, F., Bonito, G., Anslan, S., Abell, S., and Abarenkov, K. (2014). Global diversity and geography of soil fungi. *Science*, 346:6213.

Terstappen, L. W. and Levin, J. (1992). Bone marrow cell differential counts obtained by multidimensional flow cytometry. *Blood Cells*, 18(2):311–330.

The Japanese Society of Pedodontics (1988). The chronology of deciduous and permanent dentition in Japanese children. *Japanese J. Pediatr. Dent.*, 26:1–18 (in Japanese).

Thomsen, P. F., Kielgast, J., Iversen, L. L., Wiuf, C., Rasmussen, M., Gilbert, M. T. P., Orlando, L., and Willerslev, E. (2012). Monitoring endangered freshwater biodiversity using environmental DNA. *Mol. Ecol.*, 21(11):2565–2573.

Tozawa, Y., Teraishi, M., Sasaki, T., Sonoike, K., Nishiyama, Y., Itaya, M., Miyao, A., and Hirochika, H. (2007). The plastid sigma factor SIG1 maintains photosystem I activity via regulated expression of the psaA operon in rice chloroplasts. *Plant J.*, 52(1):124–132.

Trueman, C. N. and Martill, D. M. (2002). The long-term survival of bone: The role of bioerosion. *Archaeometry*, 44(3):371–382.

Tsuge, R. (1802). *Man'nanroku (in Japanese).*

Tsutaya, T., Nagaoka, T., Kakinuma, Y., Kondo, O., and Yoneda, M. (2016). The diet of townspeople in the city of Edo: carbon and nitrogen stable isotope analyses of human skeletons from the Ikenohata-shichikencho site. *Anthropol. Sci.*, 124(1):17–27.

Tsutaya, T., Nagaoka, T., Sawada, J., Hirata, K., and Yoneda, M. (2014). Stable isotopic reconstructions of adult diets and infant feeding practices during urbanization of the city of Edo in 17th century Japan. *Am. J. Phys. Anthropol.*, 153(4):559–569.

Tsutaya, T. and Yoneda, M. (2015). Reconstruction of breastfeeding and weaning practices using stable isotope and trace element analyses: A review. *Am. J. Phys. Anthropol.*, 156(S59):2–21.

Ubelaker, D. (1978). *Human skeletal remains. Excavation, analysis, interpretation.* Taraxacum, Washington.

Uhlén, M., Fagerberg, L., Hallström, B. M., Lindskog, C., Oksvold, P., Mardinoglu, A., Sivertsson, Å., Kampf, C., Sjöstedt, E., Asplund, A., Olsson, I., Edlund, K., Lundberg, E., Navani, S., Szigyarto, C. A.-k., Odeberg, J., Djureinovic, D., Takanen, J. O., Hober, S., Alm, T., Edqvist, P.-h., Berling, H., Tegel, H., Mulder, J., Rockberg, J., Nilsson, P., Schwenk, J. M., Hamsten, M., Feilitzen, K. V., Forsberg, M., Persson, L., Johansson, F., Zwahlen, M., Heijne, G. V., Nielsen, J., and Pontén, F. (2015). Tissue-based map of the human proteome. *Science*, 347(6220):1260419–1260419.

Ungar, P. S., Grine, F. E., Teaford, M. F., and Pérez-Pérez, A. (2001). A review of interproximal wear grooves on fossil hominin teeth with new evidence from Olduvai Gorge. *Arch. Oral Biol.*, 46(4):285–292.

Valentin, J. and Streffer, C. (2002). Basic anatomical and physiological data for use in radiological protection: Reference values - ICRP Publication 89. *Ann. ICRP*, 32(3-4):1–265.

Valentini, A., Pompanon, F., and Taberlet, P. (2009). DNA barcoding for ecologists. *Trends Ecol. Evol.*, 24(2):110–117.

Valentini, A., Taberlet, P., Miaud, C., Civade, R., Herder, J., Thomsen, P. F., Bellemain, E., Besnard, A., Coissac, E., Boyer, F., Gaboriaud, C., Jean, P., Poulet, N., Roset, N., Copp, G. H., Geniez, P., Pont, D., Argillier, C., Baudoin, J. M., Peroux, T., Crivelli, A. J., Olivier, A., Acqueberge, M., Le Brun, M., Møller, P. R., Willerslev, E., and Dejean, T. (2016). Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding. *Mol. Ecol.*, 25(4):929–942.

Vestheim, H. and Jarman, S. N. (2008). Blocking primers to enhance PCR amplification of rare sequences in mixed samples - a case study on prey DNA in Antarctic krill stomachs. *Front. Zool.*, 5:12.

Wang, S. Y., Cappellini, E., and Zhang, H. Y. (2012). Why collagens best survived in fossils? Clues from amino acid thermal stability. *Biochem. Biophys. Res. Commun.*, 422(1):5–7.

Warinner, C., Hendy, J., Speller, C., Cappellini, E., Fischer, R., Trachsel, C., Arneborg, J., Lynnerup, N., Craig, O. E., Swallow, D. M., Fotakis, A., Christensen, R. J., Olsen, J. V., Liebert, A., Montalva, N., Fiddyment, S., Charlton, S., Mackie, M., Canci, A., Bouwman, A., Rühli, F., Gilbert, M. T. P., and Collins, M. J. (2014a). Direct evidence of milk consumption from ancient human dental calculus. *Sci. Rep.*, 4:7104.

Warinner, C., Rodrigues, J. F. M., Vyas, R., Trachsel, C., Shved, N., Grossmann, J., Radini, A., Hancock, Y., Tito, R. Y., Fiddyment, S., Speller, C., Hendy, J., Charlton, S., Luder, H. U., Salazar-García, D. C., Eppler, E., Seiler, R., Hansen, L. H., Castruita, J. A. S., Barkow-Oesterreicher, S., Teoh, K. Y., Kelstrup, C. D., Olsen, J. V., Nanni, P., Kawai, T., Willerslev, E., von Mering, C., Lewis, C. M., Collins, M. J., Gilbert, M. T. P., Rühli, F., and Cappellini, E. (2014b). Pathogens and host immunity in the ancient human oral cavity. *Nat. Genet.*, 46(4):336–344.

Warinner, C., Speller, C., and Collins, M. J. (2015). A new era in palaeomicrobiology: prospects for ancient dental calculus as a long-term record of the human oral microbiome. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.*, 370(1660):20130376.

Welker, F., Collins, M. J., Thomas, J. A., Wadsley, M., Brace, S., Cappellini, E., Turvey, S. T., Reguero, M., Gelfo, J. N., Kramarz, A., Burger, J., Thomas-oates, J., Ashford, D. A., Ashton, P. D., Rowsell, K., Porter, D. M., Kessler, B., Fischer, R., Baessmann, C., Kaspar, S., Olsen, J. V., Kiley, P., Elliott, J. A., Kelstrup, C. D., Mullin, V., Hofreiter, M., Willerslev, E., Hublin, J.-j., Orlando, L., Barnes, I., and Macphee, R. D. E. (2015). Ancient proteins resolve the evolutionary history of Darwin's South American ungulates. *Nature*, 522(7554):81–84.

Wesolowski, V., Ferraz Mendonça de Souza, S. M., Reinhard, K. J., and Ceccantini, G. (2010). Evaluating microfossil content of dental calculus from Brazilian sambaquis. *J. Archaeol. Sci.*, 37(6):1326–1338.

Weyrich, L. S., Dobney, K., and Cooper, A. (2015). Ancient DNA analysis of dental calculus. *J. Hum. Evol.*, 79:119–124.

Weyrich, L. S., Duchene, S., Soubrier, J., Arriola, L., Llamas, B., Breen, J., Morris, A. G., Alt, K. W., Caramelli, D., Dresely, V., Farrell, M., Farrer, A. G., Francken, M.,

Gully, N., Haak, W., Hardy, K., Harvati, K., Held, P., Holmes, E. C., Kaidonis, J., Lalueza-Fox, C., de la Rasilla, M., Rosas, A., Semal, P., Soltysiak, A., Townsend, G., Usai, D., Wahl, J., Huson, D. H., Dobney, K., and Cooper, A. (2017). Neanderthal behaviour, diet, and disease inferred from ancient DNA in dental calculus. *Nature*, 544:357–361.

White, D. J. (1997). Dental calculus: recent insights into occurrence, formation, prevention, removal and oral health effects of supragingival and subgingival deposits. *Eur. J. Oral Sci.*, 105(5):508–522.

White, T. J., Bruns, S., Lee, S., and Taylor, J. (1990). *Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics*. Academic Press, San Diego.

Willerslev, E., Davison, J., Moora, M., Zobel, M., Coissac, E., Edwards, M. E., Lorenzen, E. D., Vestergård, M., Gussarova, G., Haile, J., Craine, J., Gielly, L., Boessenkool, S., Epp, L. S., Pearman, P. B., Cheddadi, R., Murray, D., Bråthen, K. A., Yoccoz, N., Binney, H., Cruaud, C., Wincker, P., Goslar, T., Alsos, I. G., Bellemain, E., Brysting, A. K., Elven, R., Sønstebø, J. H., Murton, J., Sher, A., Rasmussen, M., Rønn, R., Mourier, T., Cooper, A., Austin, J., Möller, P., Froese, D., Zazula, G., Pompanon, F., Rioux, D., Niderkorn, V., Tikhonov, A., Savvinov, G., Roberts, R. G., MacPhee, R. D. E., Gilbert, M. T. P., Kjær, K. H., Orlando, L., Brochmann, C., and Taberlet, P. (2014). Fifty thousand years of Arctic vegetation and megafaunal diet. *Nature*, 506(7486):47–51.

Wilson, J. M., Ashton, B., and Triffitt, J. T. (1976). The interaction of a component of bone organic matrix with the mineral phase. *Calcif. Tissue Res.*, 22(Supplement 1):458–460.

Ximénez-Fyvie, L. a., Haffajee, a. D., and Socransky, S. S. (2000). Microbial composition of supra- and subgingival plaque in subjects with adult periodontitis. *J. Clin. Periodontol.*, 27(10):722–732.

Yamamoto, M. (1989). Enamel hypoplasia in the deciduous teeth of Edo Japanese. *J. Anthropol. Soc. Nippon*, 97(4):475–482.

Young, J. M., Weyrich, L. S., and Cooper, A. (2014). Forensic soil DNA analysis using high-throughput sequencing: A comparison of four molecular markers. *Forensic Sci. Int. Genet.*, 13:176–184.

Ziesemer, K. A., Mann, A. E., Sankaranarayanan, K., Schroeder, H., Ozga, A. T., Brandt, B. W., Zaura, E., Waters-Rist, A., Hoogland, M., Salazar-García, D. C., Aldenderfer, M., Speller, C., Hendy, J., Weston, D. A., MacDonald, S. J., Thomas,

G. H., Collins, M. J., Lewis, C. M., Hofman, C., and Warinner, C. (2015). Intrinsic challenges in ancient microbiome reconstruction using 16S rRNA gene amplification. *Sci. Rep.*, 5:16498.

Zimmermann, E. A., Schaible, E., Bale, H., Barth, H. D., Tang, S. Y., Reichert, P., Busse, B., Alliston, T., Ager, J. W., and Ritchie, R. O. (2011). Age-related changes in the plasticity and toughness of human cortical bone at multiple length scales. *Proc. Natl. Acad. Sci.*, 108(35):14416–14421.