A53 DNA Preservation in Bone From a Multi-Individual Burial

Alexandra L. Emmons, MA, University of Tennessee, Knoxville, TN 37920; Sarah W. Keenan, PhD, South Dakota School of Mines & Technology, Rapid City, SD 57701; Jon Davoren, Lorton, VA 22079-2625; Jennifer M. DeBruyn, PhD, University of Tennessee, Knoxville, TN 37996; Amy Z. Mundorff, PhD, University of Tennessee - Anthropology, Knoxville, TN 37996-1525*

Learning Overview: After attending this presentation, attendees will understand patterns of skeletal DNA variation in buried remains and the factors that affect these patterns. This presentation will increase attendees' knowledge of human decomposition processes, including bone decay, particularly as it relates to the soil environment.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by furthering the knowledge of skeletal DNA degradation from buried environments.

There are three primary mechanisms to bone decay: (1) chemical deterioration of the organic phase of bone; (2) chemical deterioration of the inorganic phase; and (3) biodegradation. If the organic component degrades by either chemical or biological mechanisms, the mineral component, hydroxyapatite, becomes more vulnerable to environmental fluctuations and dissolution of the lattice structure.[1] This interdependence seems to suggest that greater porosity would allow for increased DNA degradation.[1,2] DNA sampling strategies and limited research has supported and emphasized this point, promoting the use of long, dense, cortical bones for DNA sampling and analysis. The preferential sampling of long bones has not only been related to DNA preservation but also to the ability to re-associate these bones in a commingled environment.[3-6] A growing body of research has indicated that small, cancellous bones outperform cortical bones in DNA quality and quantity.[6-8] The ease of sampling and reduced risk of contamination lends support to the use of smaller bones in certain cases, including disaster victim identification and may show utility in the case of a single individual burial.[9,10]

In a study looking at both intra-individual and inter-individual variation in human skeletal DNA preservation during surface decomposition, Mundorff and Davoren found that small cancellous bones, on average, yielded greater amounts of better quality DNA than larger, denser cortical bones, a pattern that held true up to 21 years postmortem.[8] The extent to which this pattern persisted in a burial remained unclear. The purpose of this research was to understand patterns of intra-individual and inter-individual skeletal DNA preservation in a multi-individual burial and how these patterns are shaped by burial factors, including soil geochemistry and biology. Three individuals were interred for four years prior to all analyses. Soil samples were collected during disinterment at four depths surrounding the bodies within the grave to look at changes in soil biology and geochemistry. To represent all bone types, a total of 49 bones per skeleton were sampled once for DNA analysis. To understand variation in human DNA quality and quality from a single bone, 19 bones per individual were sampled from two sites, and three bones were sampled from three sites. Skeletal DNA was extracted, quantified, and globalfiler results were produced from each sampling site. Co-extracted bacterial and fungal DNA was also quantified, and bacterial communities were sequenced using targeted metagenomics of the V4 region of the 16SrRNA gene using Next Generation Sequencing (NGS).

Preliminary results indicate that the small, cancellous bones of the feet outperformed other bones in DNA yield. The third cuneiform, in particular, was among the top ten highest-ranking bones in terms of DNA yield across all three individuals. DNA yield varied by individual and depth within the grave, with the shallowest individual demonstrating the highest DNA yields. The feet exhibited the greatest variation in DNA yield across bone type and sampling site. Microbial communities were significantly different by individual and sample type (bone vs. soil) using a Permutational Analysis of Variance (PERMANOVA) on unweighted unifrac distances (n=263, p=0.001). Preliminary random forest models suggest that Amplified Sequence Variants (ASVs) (microbial taxa) influence human DNA yield when data from all individuals were combined (r^2= 0.78, p=0.00).

This study provides a characterization of patterns of skeletal DNA preservation within a multi-individual grave. Results provide novel insights into skeletal DNA degradation that will likely inform skeletal DNA sampling strategies for buried remains.

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Skeletal DNA, Taphonomy, Microbial Ecology