B181 The Short-Term Effects of Surface and Subsurface Burial on DNA From Human Skeletal Remains

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After attending this presentation, attendees will understand how mitochondrial and nuclear DNA quantities vary within the human femur and how they are affected by short-term soil surface exposure and burial.

This presentation will impact the forensic science community by providing insight into both the variable levels of DNA throughout human femora and the differential degradation after short-term surface and subsurface burial.

Forensic biologists utilize human skeletal remains as a source of DNA for identification purposes. In particular, the femur and other weight-bearing long bones are commonly sampled at the midshaft diaphysis for DNA analysis in a forensic setting; however, Mundorff and Davoren demonstrated that DNA quantities vary throughout the human skeleton, with cancellous bones such as the patella and tarsals producing greater profiling success than the midshaft of weight-bearing long bones, although only one location on each bone was tested.[1,2] Previous research conducted at Michigan State University using bovine and porcine models demonstrated that DNA quantity and quality vary widely within a femur, with higher DNA yields at the epiphyses than at the diaphyses.[3] In contrast to these animal models, DNA variation within human bones has yet to be rigorously examined.

Following death, skeletal DNA begins to degrade, which can be exacerbated by unfavorable environmental factors such as temperature, moisture, pH, and burial conditions. Over time, this results in reduced DNA yields, potentially to a point where DNA is no longer retrievable; however, exactly when and to what extent DNA degradation occurs during the taphonomic process is unresolved. The goal of this study was to establish the short-term effects of surface and subsurface burial on human femoral DNA throughout the length of the bone.

Three unpreserved human bilateral femur pairs were obtained. The majority of soft tissue was removed manually, and the bones were subsequently macerated in a 0.5% Terg-a-zyme® solution. A Dremel® tool with a cobalt drill bit was bleached and Ultraviolet (UV) -irradiated and used to drill five locations on each femur (midshaft, proximal and distal diaphysis, femoral neck, and patellar groove). Bone powder was collected and its mass recorded. One femur was buried in approximately 30cm of soil, while its counterpart was placed on top of the soil, exposed to the elements. A large dog crate with the bottom removed was staked to the ground in order to protect the remains from scavengers. Femora were retrieved from the site after seven days and resampled. The powder was digested in a demineralization buffer and a 1:1 phenol-chloroform extraction was performed, followed by Amicon® column filtration. DNA extract volumes were measured prior to storage at -20°C.[4]

Mitochondrial DNA quantities at the five femoral locations were examined using an in-house, human-specific, TaqMan® assay. An internal positive control template was included to assess any Polymerase Chain Reaction (PCR) inhibition. A Quantifiler® Human DNA Quantification Kit was used to assess nuclear DNA quantity. For both mitochondrial and nuclear assays, samples that demonstrated signs of inhibition were diluted tenfold and re-tested.

Results demonstrate that prior to surface exposure or subsurface burial, mitochondrial DNA quantity was highest in the more distal femoral regions (distal diaphysis and patellar groove), whereas nuclear DNA quantity was

greater at the proximal end (neck and proximal diaphysis). Both surface and subsurface burial usually resulted in sharp decreases in DNA yields, sometimes by as much as 90%, even after only seven days. Bones that were placed on the soil surface had overall higher mitochondrial and nuclear DNA yields throughout the bone than did their buried counterparts. Finally, the proximal diaphysis maintained higher mitochondrial and nuclear DNA quantities after surface and subsurface burials than did the more peripheral regions.

In conclusion, short-term burial can have a strong and extremely rapid degrading effect on DNA from human bone, the extent of which varies across the length of the femur. Contrary to conventional thinking, the midshaft diaphysis generally does not have higher DNA yields before or after burial. Owing to this, the midshaft diaphysis should not be viewed as the optimal location for DNA recovery from human femora.

Reference(s):

1. Missing People, DNA Analysis and Identification of Human Remains. *ICRS.* 2009.
2. Mundorff A.Z., Davoren J.M. Examination of DNA Yield Rates for Different Skeletal Elements at Increasing Post Mortem Intervals. *Forensic Science International: Genetics.* 8:55-63.
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4. Loreille O.M., Diegoli T.M., Irwin J.A., et al. (2007) High efficiency DNA extraction from bone by total demineralization. *Forensic Sci Int Genet.* 1:191–5.

DNA Quantity in Bone, Skeletal DNA and Burial, DNA Degradation