B130 Intra-Bone Variation of Recoverable Nuclear and Mitochondrial DNA in Femora

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After attending this presentation, attendees will learn: (1) that DNA content varies significantly along the length of the femur; (2) the implications this has on recovering nuclear and mitochondrial DNA from skeletal remains; and, (3) practical recommendations for improving DNA recovery from femoral tissue.

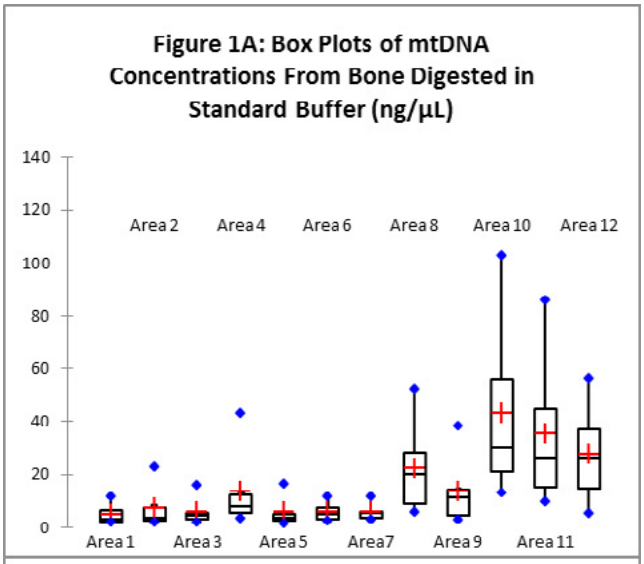
This presentation will impact the forensic science community by informing practitioners about intra-bone variation of recoverable DNA from femora. Further, these findings could have immediate impact for practitioners by improving sampling strategies, increasing first-pass success rates of generating a DNA profile, and saving forensic laboratories time, money, and resources.

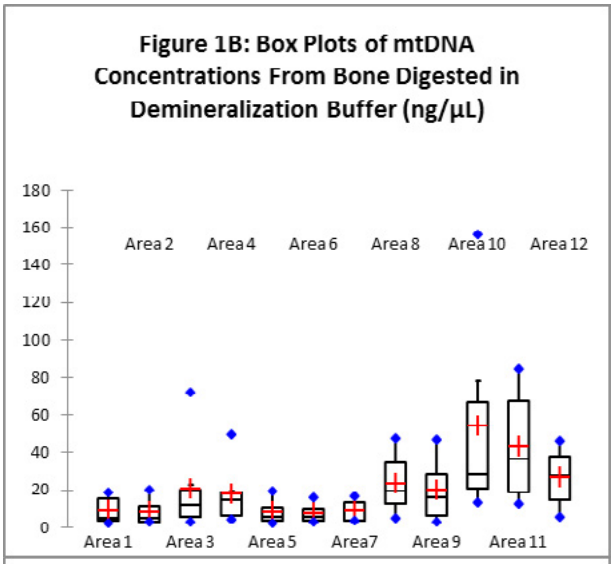
Positive identification of skeletal remains is not always possible owing to a lack of antemortem records, their level of preservation, or the remains being substantially incomplete or fragmented. Identification in these instances frequently relies on obtaining DNA from skeletal material. Research and practice have shown that successfully recovering DNA from bone is based upon several factors, including environmental conditions, extraction methodology, and different bones of the body.[1-3] Further, cortical osseous tissue from weight-bearing long bones (e.g., the femur and tibia) tends to be a better target for obtaining amplifiable DNA than trabecular bone and because midshaft diaphysis of the femur and tibia contain an abundance of cortical bone, they have become a preferential target for DNA extraction; however, there is currently a lack of research on intra-bone DNA variation.[1,4] As an example, the femur, with its large size, surface area, and varied distribution of osseous tissue types, has the potential to contain a wide array of DNA variability

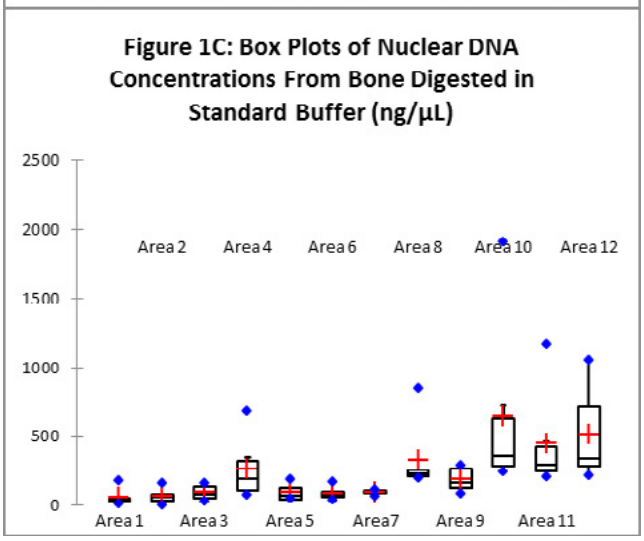
In this research, fresh bovine femora were first macerated in a boiling 1% Terg-a-zyme® solution to remove soft tissues. Twelve regions of the femur, including eight equidistant sections of diaphysis extending proximally and distally from the midshaft as well as the proximal and distal epiphyses were drilled using a Dremel® tool with a 7/64-inch cobalt drill bit. Bone powder from each region was divided between two digestion methods: standard laboratory digestion buffer (20mM Tris, — pH 7.5; 50mM EDTA; 0.1% SDS) or demineralization buffer (0.5 M EDTA — pH 8.0; 1% Laural-Sarcosinate), extracted organically, and concentrated using Amicon® filtration columns. DNA quantification of extracts utilized a TaqMan® real-time polymerase chain reaction assay targeting the nuclear MC1R and mitochondrial ATPase8 loci. To determine quality, DNAs were amplified utilizing a series of primer sets that generated ~200, 400, 600, and 1,000 base pair amplicons. All results were examined statistically at an α of 0.05 utilizing analysis of variance in conjunction with post-hoc pairwise comparisons to determine areas of significant difference.

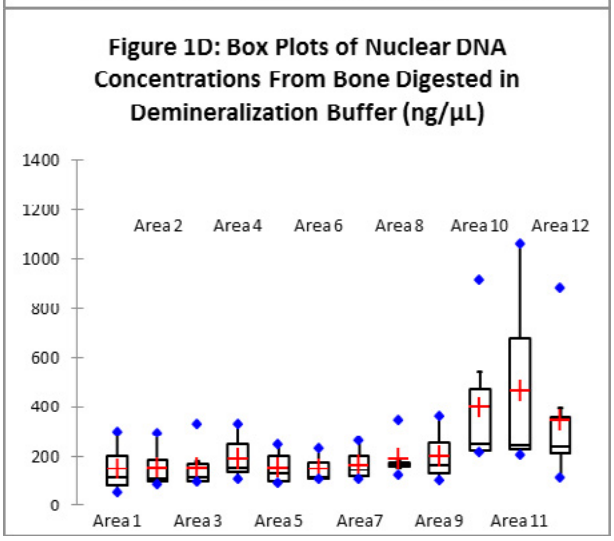
Resulting data (Figures 1A–D) showed substantial variation in DNA yields across femoral regions. Significantly more nuclear and mitochondrial DNA was obtained from the distal and proximal femoral epiphyses than the diaphysis. Areas of diaphysis close to the epiphyses, as well as auricular surfaces, had more DNA than midshaft diaphysis, which consistently had the lowest amount of recoverable DNA. The mitochondrial (1,000 base pairs) and nuclear (400 base pairs) DNA amplicons were consistently generated from all regions of the femur; however, larger nuclear DNA amplicons (600 and 1,000 base pairs) were inconsistently obtained across the femur.

These findings indicate that substantial variation in DNA levels exists along the femur. Furthermore, midshaft femur, commonly sampled by forensic practitioners, appears to be suboptimal for obtaining recoverable nuclear and mitochondrial DNA. Given the value of DNA in establishing the identity of skeletal remains and the common use of weight-bearing long bones such as the femur for obtaining that DNA, this research has the potential to be of considerable utility to forensic laboratories, and thus, to the criminal justice system.









Legend:

Area 1 (Midshaft Diaphysis)
Area 2 (Diaphysis Proximal to Area 1)
Area 3 (Diaphysis Proximal to Area 2)
Area 4 (Proximal Metaphysis)
Area 5 (Midshaft Diaphysis Distal to Area 1)
Area 6 (Diaphysis Distal to Area 5)
Area 7 (Diaphysis Distal to Area 6)
Area 8 (Distal Posterior Auricular Surface)
Area 9 (Distal Metaphysis)
Area 10 (Articular Surface of Distal Epiphysis)
Area 11 (Femoral Head)
Area 12 (Greater Trochanter)

References:

1. Edson et al. (2004). *Forensic Science Review*. 16(1):63–90.
2. Loreille et al. (2007). *Forensic Science International: Genetics*. 1(2):191–5.
3. Mundorff et al. (2013). *National Institute of Justice*. Technical Report. Award Number 2010-DN-BX-K229.
4. Misner et al. (2009). *Journal of Forensic Sciences*. 54(4):822–8.

DNA Quality and Quantity, Skeletal Variation, DNA Recovery

** Presenting Author*