H28 The Effect of Heat Associated With Maceration on DNA Preservation in Skeletal Remains

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The goal of this presentation is to present to the forensic anthropological community the differences in skeletal CNA preservation before and after exposure to heat by maceration of soft tissue.

Genetic analysis is becoming an important aspect of the individual identification process in the field of forensic science. The discovery that DNA can be extracted from skeletal remains is becoming significant to forensic anthropologists because it complements identification by osteological analysis. The maceration of soft tissue is an important step in skeletal analysis because the removal of soft tissue makes signs of trauma and individualizing characteristics easier to see. However, the same taphonomic forces that affect bone preservation also affect the preservation of the DNA contained within the skeletal material. Heat is a factor that is known to increase the degradation process by accelerating the rate of the chemical reactions responsible for human decomposition. This poster will present the differences in skeletal DNA preservation before and after the process of removing soft tissue by boiling, and show whether these differences are significant in obtaining the amount of DNA needed for genetic analysis.

For genetic analysis, 15 *Sus scrofa* (pig) femora were sampled before and after 6 hours of simmering in water, 70 ml of Borax and 100 ml of bleach at 95°C. An additional 13 pig femora were sampled for genetic analysis before and after 10 hours of simmering in water, 70 ml of Borax and 100 ml of bleach at 95°C. Water was periodically added to keep the bones submerged. Two time spans were chosen in order to investigate a wider range of heat exposure. The femora were collected from pigs displaying varying stages of natural decomposition, ranging from 1 month to 1 year of surface exposure, which would be similar to the condition of skeletal remains that a forensic anthropologist would receive for analysis. Pig remains were chosen because the decomposition process and bone microstructural make-up are similar to that of humans, and because specimens are easily obtained for forensic studies.

A pig specific DNA probe was used to determine DNA quantification by luminescent detection using CDP-Star chemiluminescent techniques. Chemiluminescent detection relies on probes designed to bind to the DNA region of interest. Primers for a pig specific DNA sequence of 374 base pairs in length, a portion of the Pre-1 porcine short interspersed nuclear element, were designed to allow abundant amplification of the target DNA sequence. Short and long interspersed nuclear elements comprise over a third of the genome in higher eukaryotes and are common target sites for PCR based genetic fingerprinting. The Pre-1 porcine short interspersed nuclear element is found on several pig chromosomes at a relatively high copy number, and provides a tool for indirectly quantifying DNA recovered from pig bones. The Pre-1 DNA probe was produced by PCR amplification of DNA isolated from the white blood cells of freshly drawn pig blood obtained from a local veterinarian. The PCR product was crosslinked to an alkaline phosphatase enzyme that converts a dioxetane phosphate substrate to a dioxetane product. This conversion emits photons of light, which are captured on X-Ray film and used to determine the amount of DNA in the sample. A greater luminescent intensity indicates a larger amount of DNA. Known amounts of DNA from the pig white blood cells were quantified and used to construct a standard curve by slot blot analysis. The skeletal DNA samples were then quantified by slot blot analysis and compared to the standard curve for analysis.

Temperature increases are known to accelerate the degradation rate of DNA by destabilizing hydrogen bonds, and increase the probability of strand breaks and cross-link formation. Comparing the amount of DNA obtained before and after maceration is of value to forensic anthropologists in demonstrating if the heat generated by the boiling of skeletal remains is great enough to affect the amount of skeletal DNA available for future genetic analysis.

Skeletal DNA, Maceration, Genetic Analysis

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