H26 The Ability to Amplify Skeletal DNA After Heat Exposure Due to Maceration

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After attending this presentation, attendees will become aware that the heat generated by the boiling of skeletal remains during maceration does not adversely affect the ability to amplify and analyze skeletal DNA found in the bone samples.

Forensic anthropologists can take bone samples for future DNA amplification (via the polymerase chain reaction) and analysis before or after removing soft tissues by the process of maceration. The heat and chemicals associated with the maceration process do not hamper the ability to successfully extract and amplify skeletal DNA found within the bone.

The goal of this presentation is to present to the forensic anthropological community the effect of heat exposure, due to the maceration of soft tissues, on subsequent polymerase chain reaction (PCR) analysis of skeletal DNA.

The sampling of skeletal material for genetic analysis is becoming increasingly common in the forensic sciences. For forensic anthropologists, a genetic profile obtained from skeletal DNA complements the biological profile constructed for an individual. In order to conduct a thorough skeletal analysis it is often necessary to remove by maceration any soft tissues that may conceal signs of trauma or bony landmarks used to create the biological profile. The maceration process involves immersing the remains in simmering water for an extended period of time. Because both heat and water are known to increase the rate of DNA degradation, and therefore hamper DNA amplification and analysis it is reasonable to think maceration may complicate the ability to successfully extract and amplify skeletal DNA found within the bone. However, the authors have previously demonstrated that the maceration of skeletal remains can improve the amount of extracted skeletal DNA detectable by chemiluminescent techniques, although the quantity of extracted DNA does not predict the quality or ability to amplify the DNA. This poster will present a subsequent study comparing the differences in the ability to amplify skeletal DNA before and after the process of removing soft tissue by maceration, and show that these differences are insignificant via statistical analysis.

Femora were collected from *Sus scrofa* (pigs) displaying varying stages of natural decomposition, ranging from one month to one year of surface exposure in a wooded area, which would be similar to the condition of skeletal remains that a forensic anthropologist would receive for analysis. Pig remains were chosen because their bone decomposition and microstructure are similar to that of humans, and because specimens are easily obtained for experimental studies. Fifteen pig femora were sampled for genetic analysis before and after six hours of simmering in water, 70 ml of Borax and 100 ml of bleach (in a 50cmx31cmx15cm pan) at 95º C. An additional thirteen pig femora were sampled for genetic analysis before and after ten hours of simmering in water, 70 ml of Borax and 100 ml of bleach (in a 50cmx31cmx15cm pan) at 95º C. Time intervals of six and ten hours were chosen in order to investigate a wider range of heat exposure. Water was periodically added to keep the bones submerged and keep the Borax and bleach concentrations constant.

Short interspersed nuclear elements (SINEs) are common target sites for PCR based genetic fingerprinting because of their high copy number in humans and other higher eukaryotes. A pig specific primer was designed to amplify a 374 base-pair region of the Pre-1 porcine SINE, which is found on several pig chromosomes. The Pre-1 porcine SINE primers were added to the skeletal DNA samples and amplified via PCR consisting of 35 consecutive cycles at 95º C for one minute, 62º C for one minute, and 72º C for one minute. The amplified samples were electrophoresed through an agarose gel and visualized after immersion in ethidium bromide and exposure to ultra-violet light. Amplification success was determined by the presence or absence of amplified skeletal DNA visible on the agarose gel. A total of 15 out of the 28 samples taken before maceration contained amplifiable DNA and 18 out of the 28 samples taken after maceration contained amplifiable DNA. The number of samples containing amplifiable DNA before versus after maceration was compared using McNemar's test for significant changes showed no significant difference between the two groups of samples at p=0.05.

High temperatures are known to increase the rate of DNA degradation by disrupting the chemical bonds in DNA molecules and accelerating fragmentation rates. This investigation into the amplification success of skeletal DNA obtained before versus after maceration is of value to forensic anthropologists in demonstrating that the heat generated by the boiling of skeletal remains does not adversely affect the ability to amplify and analyze DNA found in the samples.

Skeletal DNA, PCR, Maceration

 ** Presenting Author*