H23 Impact of Heat and Chemical Maceration on DNA Recovery and Cut Mark Analysis

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The goals of this presentation are to educate forensic anthropologists and pathologists about the impact of common maceration techniques on the interpretation of tool marks and recovery of DNA from human bone.

Molecular genetics is a powerful tool in the forensic sciences, with bones increasingly utilized as sources of both genomic and mitochondrial DNA. Thus, anthropologists must be extraordinarily cognizant of how skeletal materials are handled and chemically treated while in their custody. Few jurisdictions require bone samples to be extracted prior to anthropological analysis, and there are instances in which a case review at a later point in time may require molecular identification. Although chemical preservatives, such as polyvinyl acetate (PVA), are no longer in wide usage among forensic anthropologists because of the deleterious affects on DNA quality, there are no studies known to the authors that examine the impact of an even more widespread anthropological protocol – maceration. Anthropologists employ a varying array of chemicals and temperatures in maceration protocols but the affects on DNA quality and quantity are currently unknown. In addition, the anthropologist must always be concerned about the impact of soft tissue maceration on the subtle signs of sharp trauma. Since sharp trauma may leave only superficial marks in bone, it is possible that chemical stripping of the periosteum may compromise the appearance of incisions and/or create postmortem damage that may complicate anthropological analysis. Therefore, the purpose of this study is to evaluate the affects of common maceration techniques on DNA degradation and cut mark integrity.

In order to quantify the amount of mitochondrial and genomic DNA from both cortical and spongy bone, fresh pig (*Sus scrofa*) rib cage sections and upper forelimbs were utilized. Bone samples were taken for DNA analysis before and after maceration. To test cut mark integrity, a single researcher stabbed each set of remains twice and marked the end of the cut bone with a notch for later reference. The remains were macerated using a control of warm water (90°C) and multiple experimental procedures found in published and non-published sources, including: bleach solution, dish soap, and meat tenderizer, EDTA and papain, hydrogen peroxide solution, and alternative heating methods such as an incubator and microwave oven. Nonmetal tools and soft toothbrushes were used to gently remove loose tissue during the maceration process. Observations were taken of the texture of the meat and bones, odor, duration of procedure (in hours), and bone condition. The bones were allowed to air dry prior to examination.

A number of variables were scored for each experiment, including the time to complete maceration, ease of maceration, overall bone quality, and integrity of the cut mark. DNA recovery was quantified by initial spectrophotometry (A230, A260/280), followed by fluorescence-activated quantification based upon the DNA amount per bone sample. Briefly, the DNA pools from each bone sample were labeled with the fluorescent intercalating agent SYBR gold (Molecular Probes, Eugene, Oregon) and overall amount of DNA was quantified using a FLA3500 PhosphorImaging System (Fujifilm, Tokyo, Japan). While the extraction of any amount of DNA from bone is quite significant, the isolation of intact DNA is the ultimate goal. To address this vital point, two experimental methods were utilized to detect the quality of DNA in each bone sample, agarose gel electrophoresis and PCR amplification. Following extraction, intact genomic DNA will run as a single band on an agarose gel. The presence of multiple bands or smears on the gel signifies degraded DNA. PCR amplification using primers designed to two gene loci, one nuclear and one mitochondrial, were then used to confirm the quality of the extracted DNA.

The results indicate that the type of maceration technique had little affect on sharp trauma observations (though bleach was problematic) but that the DNA recovery rate was negatively influenced by excessive heat and a number of chemical treatments. The most effective maceration methods that preserved DNA also proved to be the most economical. It is recommended that forensic anthropologists use conservative, nonaggressive maceration protocols in the likely event that the bones will be utilized as a source of molecular information.

DNA, Sharp Trauma, Maceration

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