A152 An Evaluation of Mitochondrial DNA Success Rates in a Commingled Assemblage From the Cabanatuan Prisoner of War (POW) Cemetery

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Learning Overview: The objective of this presentation is to explore analyses differences in mitochondrial DNA success rates by DNA testing strategy, element type, and taphonomic modifier.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing data to aid in better selection skeletal elements for DNA sampling in forensic contexts, particularly when skeletal material is in poor condition and/or commingling is present. This study also examines how various taphonomic alterations may influence DNA success rates.

The Cabanatuan POW Camp was a World War II POW camp located in the Philippines, associated with 2,763 American casualties. Unidentified individuals were eventually interred in the Manila American Cemetery and Memorial (MACM), and historians estimate there are approximately 990 to 1,006 unresolved Cabanatuan individuals.

The Cabanatuan Project, initiated by the Defense POW/MIA Accounting Agency, is a focused effort to identify these unknowns.[2] The remains are commingled and in poor condition.[1] Although each casket presumably designates a single individual, anthropological and DNA analyses have demonstrated as many as seven individuals represented in single casket.[3]

Due to the poor condition of the Cabanatuan remains, many anthropological analyses are not applicable. In addition, the remains were treated with lye and formalin prior to burial at the MACM. Identification of individuals often hinges on DNA results. Selecting skeletal elements likely to yield DNA is crucial to this endeavor. This study examines DNA results to answer two important questions: (1) What elements are most likely to sequence?; and (2) What effect do taphonomic alterations have on DNA success rate?

The DNA results of 20 individual caskets were analyzed. Every element tested for DNA was recorded, and DNA yield was reported as successful if the Armed Forces Medical Examiner System-Armed Forces DNA Identification Laboratory reported a sequence. The type of DNA testing strategy (Sanger or Next Generation Sequencing [NGS]) used for each element was recorded. The presence of commonly observed taphonomic alterations, which included the presence of vivianite (a type of phosphate crystal) and rust staining, were visually assessed, when possible.

A total of 318 sampled elements (404 DNA tests) were included in this study. There were 121 upper limb elements, 112 lower limb elements, 61 teeth, and 19 cranial elements. The remaining elements included a few vertebrae and foot elements. There were a total of 215 Sanger tests and 189 NGS tests. Some elements were sampled more than once, and some samples, after failing to sequence in Sanger, were subsequently tested with NGS (n=86). For the purposes of this study, multiple failures of a specific test from a single element were only counted once.

Independence and goodness of fit frequency tests were used to examine differences between DNA success rates of Sanger and NGS. Overall, the difference between successful Sanger and NGS testing for the entire assemblage was slight: Sanger 41.86% (90/215) vs. NGS 41.80% (79/189). However, there were differences in DNA success rates between Sanger and NGS among body regions. NGS had greater success for teeth at 69.70% (23/33) vs. 51.28% (20/39) for teeth in Sanger (p<0.001). Sanger had a higher success rate for lower limb elements (60.27%; 44/73) vs. NGS (43.55%; 27/62) (p<0.001). There was not a significant difference between NGS and Sanger modalities for upper limb elements (p=0.134).

Taphonomic information was collected for available skeletal elements, excluding teeth (n=169). Fifty-nine elements were observed with vivianite crystals. Fifty-seven of these elements were tested in NGS and 9/57 (15.78%) sequenced. Only two elements with vivianite were tested in Sanger and neither sequenced. Rust staining was observed on 111 elements. Forty rust-stained elements were tested in Sanger and 55.00% (22/40) successfully sequenced, while only 30.99% (22/71) tested in NGS were successful (p<0.05). Forty elements had both vivianite and rust staining; however, taphonomic signatures were assessed independently.

Overall, the Sanger and NGS success rates varied within this Cabanatuan assemblage based on element type. The presence of vivianite was found to indicate poor DNA success in NGS; however, this may be correlated to other underlying factors, such as prior treatment or the waterlogged casket environment. Future studies may further examine this relationship. This information may be useful to other forensic practitioners making DNA sampling decisions for commingled, treated, or waterlogged remains.

Reference(s):

1. Megyesi M.S. 2018. Resolving Commingling and Past Accounting at Cabanatuan Prison Camp Cemetery. *Proceedings of the American Academy of Forensic Sciences*, 70th Annual Scientific Meeting, Seattle, WA. 2018.
2. Richer S.M., Megyesi M.S. 2018. Burial Location in the Manila American Cemetery and Memorial and its Relationship to Taphonomic Condition of Skeletal Remains. *Proceedings of the Canadian Association for Physical Anthropology*, October 31-November 3, 2018; London, ON, Canada.
3. Megyesi M.S. 2019. Challenges to Identifications of the Cabanatuan Prison Camp Cemetery remains. *Forensic Anthropology* 2:113-120.

DNA, Commingling, Taphonomy

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