EFFECTS OF 17 YEARS OF BURIAL IN THE RECOVERY OF GENETIC INFORMATION FROM BONE SAMPLES

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DNA analysis from bone samples is an important alternative for the identification of missing persons once these samples are often the only biological material available. Environmental conditions including temperature, humidity and soil characteristics are critical for the outcome, but the time frame of exposure to such conditions imparts greater impact at the recovery of usable DNA for genotyping.

In this work, we present a case study comparing results obtained from two skeletons, now on called AXP and IXP. Remains were first exhumed in 1996 and samples taken from the right femurs were sent to a private laboratory for DNA identification. No genetic information was obtained from AXP and partial HVI mtDNA sequences were obtained from IXP, resulting in a maternal lineage hit with reference sample taken from the alleged mother of two missing brothers. Despite the non-conclusive results at DNA examination, IXP was considered identified and so AXP by association, once both skeletons were found side by side at the grave site and investigations carried out indicated that they had been buried together.

The case was considered closed and skeletons were buried again, with the exception of fragments from the two right femurs that were kept in a room with controlled humidity and temperature for retesting.

In 2013, the family claimed the genetic identification of AXP and a confirmation of the identification of IXP. Remains were once again exhumed and new attempts of DNA identification were made at our laboratory. We had access to bone samples of left femurs taken during the second exhumation and the samples from right femurs mentioned before.

Bone samples were pulverized and DNA extraction performed by total demineralization using an internally developed protocol. DNA samples were quantified by qPCR (Quantifiler Human and Plexor®HY), submitted to mtDNA sequencing (HVI and HVII regions) and autosomal STR typing (PowerPlex®ESX17).

Dramatic differences at quantity and quality of the genetic material were observed between the samples recovered in 1996 and those recovered in 2013. No interpretable genetic information was obtained from AXP samples recovered in 2013, but full STRs profiles and partial mtDNA sequences were obtained for the samples from the same individual recovered in 1996. Partial STRs profiles and mtDNA sequences were obtained for IXP samples recovered in 1996 and 2013, but the number of loci and length of the mtDNA sequences were bigger for samples recovered in 1996. IXP and AXP were finally genetically identified as the two missing brothers that disappeared in 1972.

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