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Autosomal and Y-STR analysis of degraded DNA from the 120-year-old skeletal remains of Ezekiel Harper

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ABSTRACT

The 120-year-old skeletal remains of Confederate Civil War soldier Captain Ezekiel "Zeke" Harper were exhumed by court order in January 2011 for DNA analysis. The goal of the DNA testing was to support or refute whether Captain Harper had fathered a son (Earl J. Maxwell) with his Native American maid prior to his murder in 1892. Bones with adequate structural integrity (left tibia, right tibia, right femur, mandible, four teeth) were retrieved from the burial site and sent to the Institute of Applied Genetics in Fort Worth, Texas for analysis. Given the age and condition of the remains, three different extraction methods were used to maximize the probability of DNA recovery. The majority of the DNA isolates from over fifty separate bone sections yielded partial autosomal STR genotypes and partial Y-STR haplotypes. After comparing the partial results for concordance, consensus profiles were generated for comparison to reference samples from alleged family members. Considering the genetic recombination that occurs in autosomal DNA over the generations within a family, Y-STR analysis was determined to be the most appropriate and informative approach for determining potential kinship. Two of Earl J. Maxwell's grandsons submitted buccal samples for comparison. The Y-STR haplotypes obtained from both of these reference samples were identical to each other and to the alleles in Ezekiel Harper's consensus profile at all 17 loci examined. This Y-STR haplotype was not found in either of two major Y-STR population databases (U.S. Y-STR database and YHRD). The fact that the Y-STR haplotype obtained from Ezekiel's skeletal remains and Earl's grandsons is not found in either population database demonstrates its rarity and further supports a paternal lineage relationship among them. Results of the genetic analyses are consistent with the hypothesis that Earl J. Maxwell is the son of Ezekiel Harper.

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1. Introduction

Ezekiel "Zeke" Harper (Fig. 1) was born in Tucker County, West Virginia in 1823 [1]. His early life was spent exploring the high Alleghenies (part of the vast Appalachian Mountain Range that spans the eastern United States and Canada), working in the California gold fields, and trekking across the Rocky Mountains and Sierra Nevadas with a wagon train of "forty-niners." By the mid-1850s, he had become a prosperous landowner, cattle baron, miner, and merchant in both California and Oregon [1–3].

In 1860, on the eve of the American Civil War, Zeke returned home to West Virginia and immediately sided with the Confederacy. In contrast to the large numbers of soldiers needed to fight in the valleys or flatlands, the steep forested terrain of the Allegheny highlands favored small bands of men who could strike stealthily and then quickly disappear into the brush. Zeke knew the obscure mountain trails and thus was prized by military leaders as a potential guide and scout. Zeke and his older brother William "Devil Bill" Harper became two of the most famed Confederate guerrilla scouts in the region during the Civil War. His most notable accomplishment occurred during April–May 1863 when he led two

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Fig. 1. Photo of Captain Ezekiel "Zeke" Harper (1823–1892), Confederate guerrilla scout during the American Civil War (Photo courtesy of Maxwell family).

brigadier generals in the famous Jones-Imboden Raid, a Confederate attack which destroyed a Baltimore-and-Ohio (B&O) Railroad bridge that was vital to the Union supply lines through western Virginia [1–4]. In October 1863, Zeke was captured by Yankee soldiers and local Unionists. Over the next several months, he was imprisoned and transported between several prisoner-of-war (POW) camps, including Atheneum Prison in Virginia and Camp Chase in Ohio. In January 1864 he was sent to Illinois' notorious Rock Island Prison, where he remained until he was traded back to Confederate authorities in February 1864 [1].

Zeke survived the Civil War and returned to Tucker County, where he remained for the rest of his life. Over the years, he accumulated approximately 4500 acres of land and became a renowned country doctor [1]. Sometime between 1878 and 1888, he was rumored to have fathered a son with his Native American maid. In March 1892, Zeke was beaten to death during a robbery [2,3] and his alleged son was sent to the County Farm, a local orphanage. Sarah Bonnifield Maxwell, Zeke's girlfriend prior to the Civil War, tracked the child down at the orphanage and took him home to raise as her own. This alleged son, Earl J. Maxwell, fathered seven children during his lifetime. After Earl's death, his children and grandchildren began pursuing an investigation that could establish their familial link to Ezekiel and substantiate Earl's claim to be his son. In January 2011, the 21st Circuit Court of Tucker County, West Virginia granted an order for the disinterment of Ezekiel Harper from the Adam Harper Cemetery (Clover District, St. George, West Virginia) for the purpose of DNA testing. Ezekiel's grave was marked clearly with a well-maintained headstone, as were all of the other graves in the private family cemetery. In collaboration with the Lohr and Barb Funeral Home (Parsons, West Virginia), the exhumation of Mr. Harper's remains was conducted by the Mercyhurst Archaeological Institute (Erie, Pennsylvania), and select samples were sent to the Institute of Applied Genetics in Fort Worth, Texas for analysis.

2. Materials and methods

2.1. Preparation of skeletal elements for DNA extraction

Upon exhumation, it was discovered that Ezekiel had been buried in a wooden casket with an apparent inner glass vault/casing, both of which had deteriorated and collapsed under the weight of the soil. Bones with adequate structural integrity were retrieved from the burial site and the following were sent to the lab for analysis: left tibia, right tibia, right femur, mandible, and four teeth (2 canines, 1 lateral incisor, 1 premolar). A description of each of these skeletal elements is outlined in Table 1. Photographs are presented in Supplementary Figs. 1–3.

Prior to extraction, the external surfaces of the femur, both tibiae, and all four (4) teeth were sanded with a Dremel® 4000 High Performance Rotary Tool and individually sterilized grinding stones. Surface-sanding was conducted under a laminar flow hood in a designated low-template (LT) area of the laboratory. The mandible was not processed for DNA extraction due to its poor structural condition. After sanding, the diaphysis of the femur and both tibiae were sectioned using a Stryker® autopsy saw and individually sterilized Stryker® sectioning blades. Each resultant bone section was placed in a sterile 50 ml polypropylene conical tube. Further surface decontamination procedures were performed on individual bone sections and teeth to remove any remaining exogenous or contaminant DNA. Each bone fragment or tooth was immersed in 50% commercial bleach (3% sodium hypochlorite) for 10–15 min, followed by 4–5 washes with molecular grade (nuclease-free) water and brief immersion in 95% ethanol. After the ethanol rinse, conical tubes containing individual teeth or bone sections were placed in a PCR hood overnight to dry.

Each individual bone or tooth then was placed (along with a stainless steel impactor) in a sterile polycarbonate sample vial flanked by two stainless steel endcaps. Sample vials were submerged in the liquid nitrogen chamber of an SPEX SamplePrep 6750 Freezer Mill® and ground into a fine powder using the following cycle parameters: 10-min pre-chill, 5-min grind time, 15 impacts-per-second. Post-grinding, bone powder from each sample was transferred to sterile 15 ml polypropylene conical tubes in 0.5-g aliquots in preparation for DNA extraction.

2.2. DNA extraction methods: skeletal remains

Due to the age and condition of the remains, three different extraction methods were employed in an effort to maximize the possibility of DNA recovery. Bone samples were extracted separately in small batches in a low-template (LT) area of the laboratory.

2.2.1. Amicon® Ultra-4/MinElute® extraction

Bone samples were extracted according to the method described by Loreille et al. [5], using 0.5 g bone powder for each extraction.

2.2.2. Hi-Flow® silica column extraction

Bone demineralization was carried out by mixing 0.5 g bone powder with 3 ml digestion buffer (0.5 M EDTA pH 8.0, 1% sodium N-lauroylsarcosinate, 100 μg/ml proteinase K), followed by incubation in a hybridization oven at 56 °C under constant agitation for 24 h. After demineralization, bone powder was pelleted via centrifugation at 2545 × *g* for 5 min. The supernatant was transferred to a sterile 15 ml conical tube and mixed with five volumes of binding buffer (PB buffer, Qiagen Cat. #19066). This

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