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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

Table 1
Description of skeletal elements submitted for DNA analysis.

Skeletal element	Description/condition
Left tibia	Shaft fragment Intact lateral aspect Exposed medullary cavity
Right tibia	Proximal epiphysis absent Portion of distal epiphysis present Exposed medullary cavity
Right femur	Proximal epiphysis absent Portion of distal epiphysis and condyles present Exposed medullary cavity
Mandible	Incomplete Right ascending ramus absent Portion of posterior horizontal ramus absent Root of second or third molar present (crown absent)
Teeth	Right canine Left canine Left lateral incisor Premolar of indeterminate side and number Roots intact; crowns worn

mixture was vortexed thoroughly, transferred to a Hi-Flow® DNA Purification Spin Column (Generon, Berkshire, UK), and centrifuged at 2545 × *g* for 10 min. With the Hi-Flow® silica column, both cleanup and volume reduction were accomplished with a single device, decreasing the chances of contamination. After discarding the flow-through, the column was washed with 15 ml PE buffer (Qiagen Cat. #19065) and then centrifuged at 2545 × *g* for 5 min to remove residual ethanol. The column was transferred to a sterile collection tube and the DNA bound to the membrane was eluted with 100 μl EB buffer (Qiagen Cat. #19086).

2.2.3. Phenol–chloroform (organic) extraction

Bone demineralization was achieved by mixing 0.5 g bone powder with 3 ml digestion buffer (0.5 M EDTA pH 8.0, 1% sodium N-lauroylsarcosinate) and 200 μl of proteinase K (20 mg/ml). Samples were incubated at 56 °C under constant agitation for 24 h. After the incubation period, DNA was extracted using a standard phenol:chloroform:isoamyl alcohol (25:24:1) (PCIA) procedure followed by concentration with Amicon® Ultra-4 centrifugal filter devices (Millipore Corp., Billerica, MA). The concentrated samples then were further purified using the Qiagen QIAquick® PCR Purification Kit (Cat. #28106) following the manufacturer's recommendations.

2.3. DNA extraction: reference samples

Reference samples from alleged family members, from individuals who were present during excavation of the burial site, and from the anthropologist who performed the exhumation were collected using Sampact® Buccal Cell Sample Cards (Fitzco, Minneapolis, MN). Extractions were performed using the QIAamp DNA Investigator Kit (Qiagen, Valencia, CA) in an area of the laboratory dedicated to reference samples.

2.4. DNA quantification

The quantity of DNA in each extract was determined using the Quantifiler® Human DNA Quantification Kit (Life Technologies, Foster City, CA) and an ABI 7500 Real-Time PCR System. The assay was carried out in a 25 μl total reaction volume (23 μl Quantifiler® master mix and 2 μl DNA extract), with sample concentrations determined via comparison to a standard curve.

2.5. PCR amplification

Amplification of autosomal and Y-chromosome STRs was carried out according to the manufacturer's recommendations using AmpF*l*STR® Identifiler® Plus and AmpF*l*STR® Yfiler®, respectively (Life Technologies, Foster City, CA) [16]. Thermal cycling was performed in an ABI GeneAmp® 9700 PCR System, and all bone extracts were processed prior to amplification of reference sample DNA. Additionally, some extracts were amplified with PowerPlex® Y23 (Promega, Madison, WI) using standard reaction parameters.

2.6. DNA separation, detection, and analysis

The amplified DNA samples were prepared for electrophoresis (1 μl PCR product, 8.7 μl of Hi-Di™ Formamide, and 0.3 μl of GeneScan™ 600 LIZ® Internal Lane Size Standard). One microliter of AmpF*l*STR Identifiler® Plus or Yfiler® allelic ladder was included at least once per injection on the 96-well plate. All samples were denatured at 95 °C for 5 min and then immediately cooled on ice for 5 min. Electrophoresis was performed on an ABI 3500*xl* Genetic Analyzer (Life Technologies, Foster City, CA) with POP-4™ polymer and standard (default) injection parameters. STR data were sized and typed with GeneMapper® ID-X Software Version 1.2 (Life Technologies, Foster City, CA) with an analytical threshold of 75 RFU for Identifiler® Plus and 150 RFU for Yfiler® results.

3. Results and discussion

Skeletal remains often contain limited quantities of DNA that can be substantially degraded and copurify with environmental inhibitors. Given the age and condition of Ezekiel's remains, the same contamination controls recommended for archaeological and ancient DNA specimens were used throughout this study, including: (a) use of protective suits, gloves, and masks; (b) bleach de-contamination and UV-irradiation of work benches and associated equipment; (c) physical removal and/or chemical destruction of contaminant/exogenous DNA on external bone surfaces; (d) extraction of bone samples in a designated low-template (LT) area; (e) PCR amplification in a location that is physically separated from the extraction area; (f) use of appropriate amplification controls; and (g) replicate testing to check for reproducibility and to increase the reliability of results [6,7,10–14].

Ezekiel Harper's skeletal remains were stored separately from other samples in the LT area of the laboratory. A chain-of-custody was maintained, and the same person (a female) conducted all of the testing on the remains, including bone sectioning, DNA extractions, DNA quantification, PCR amplification, and STR typing. The same female analyst also processed all reference samples. To prevent cross-contamination from modern sources, the bones were processed and typed prior to collecting reference samples for comparison; hence, the reference samples could not be the source of the profile obtained from the skeletal remains. Additionally, reference samples were processed in a designated high-copy area of the laboratory (separate from the bones, which were extracted and analyzed in the LT area). All individuals involved in the exhumation and all laboratory personnel were excluded as possible sources of both the autosomal and Y-STR profiles that were obtained from Ezekiel's remains.

3.1. Autosomal DNA results

The AmpF*l*STR® Identifiler® Plus kit was selected for use in this project because of its higher sensitivity and improved performance with LT and inhibited samples than the earlier generation Identifiler® kit [8,9]. Partial autosomal DNA profiles were obtained

from all teeth and bone sections. No alleles were detected in any of the reagent blanks or negative controls. Alleles observed in the partial profiles from each skeletal sample were compared for concordance, and a consensus STR genotype was generated prior to reference sample processing (Supplementary Table 1). As shown in Supplementary Table 1, the alleles observed for the CSF1PO and D2S1338 loci were not included in the consensus genotype due to infrequent and inconsistent results among samples. Since an inverse relationship exists between locus size and successful PCR amplification, this observation is consistent with a number of previous studies which found that STR loci with larger sized amplicons are the first to drop out of the profile in degraded samples [18–21].

Many of the bone fragments produced during sectioning yielded sufficient bone powder for two separate extractions, which provided an opportunity to compare the efficacy of different extraction methods on these older, historical skeletal remains. However, it is possible that following grinding there could be non-uniform distribution of bone powder within the tube based upon particle size and/or density. Therefore, the first and second bone powder fractions were alternated between extraction methods to reduce possible sampling bias. Fig. 2 shows the average number of allele calls per locus for twelve bone samples (6 tibiae, 6 femora). The organic extraction method generally outperformed Hi-Flow® extraction for all 16 loci examined. Lower peak heights and fewer total observed alleles with the Hi-Flow® extracts could be due to loss of small DNA fragments during the wash step and/or retention of DNA in the Hi-Flow® column during the elution step. Hi-Flow® column DNA recovery percentages have been reported in the range of 80–95% [15]; however, DNA samples that are severely degraded and/or that already exist in low quantities when received for analysis may be more problematic. Regardless of the cause of the differences in the number of alleles observed between these two extraction methods, organic extraction performed better with these older skeletal remains. A comparison between the Amicon® Ultra-4/MinElute® and Hi-Flow® extraction methods using 32 bone samples (16 tibiae, 16 femora) demonstrated only minimal differences in allele recovery per locus (Fig. 3). A direct comparison between the organic and Amicon® Ultra-4/MinElute® methods could not be performed due to lack of sufficient bone powder for testing.

3.2. Y-chromosome (Y-STR) results

Seventeen loci on the Y-chromosome were examined using extracts from four teeth, 23 femur sections, and 22 tibia samples. Partial Y-STR profiles were obtained with the majority of the samples, and only seven bone sections (3 femora, 4 tibiae) yielded no results. No alleles were detected in any of the reagent blanks or negative controls. The alleles observed in the partial profiles from each sample were compared for concordance, and a consensus Y-STR haplotype was generated with the AmpF*l*STR® Yfiler® 17-loci multiplex kit (Supplementary Table 2). Concordance in allele calls was further supported with extracts from three arbitrarily selected samples (one tooth, one femur section, one tibia section) that were amplified using the PowerPlex® Y23 kit (Promega, Madison, WI). Seventeen of the 23 loci amplified with PowerPlex® Y23 overlap with the Yfiler® markers. Allele calls generated for the same loci between the two kits were consistent, and for these particular samples the PowerPlex® Y23 kit outperformed AmpF*l*STR® Yfiler® in terms of total number of alleles recovered (Table 2).

Bone fragments that yielded sufficient bone powder for two separate extractions were used to compare the efficacy of different extraction methods, and the first and second powder fractions were alternated between extraction methods to reduce potential sampling bias. Fig. 4 shows the average number of allele calls per

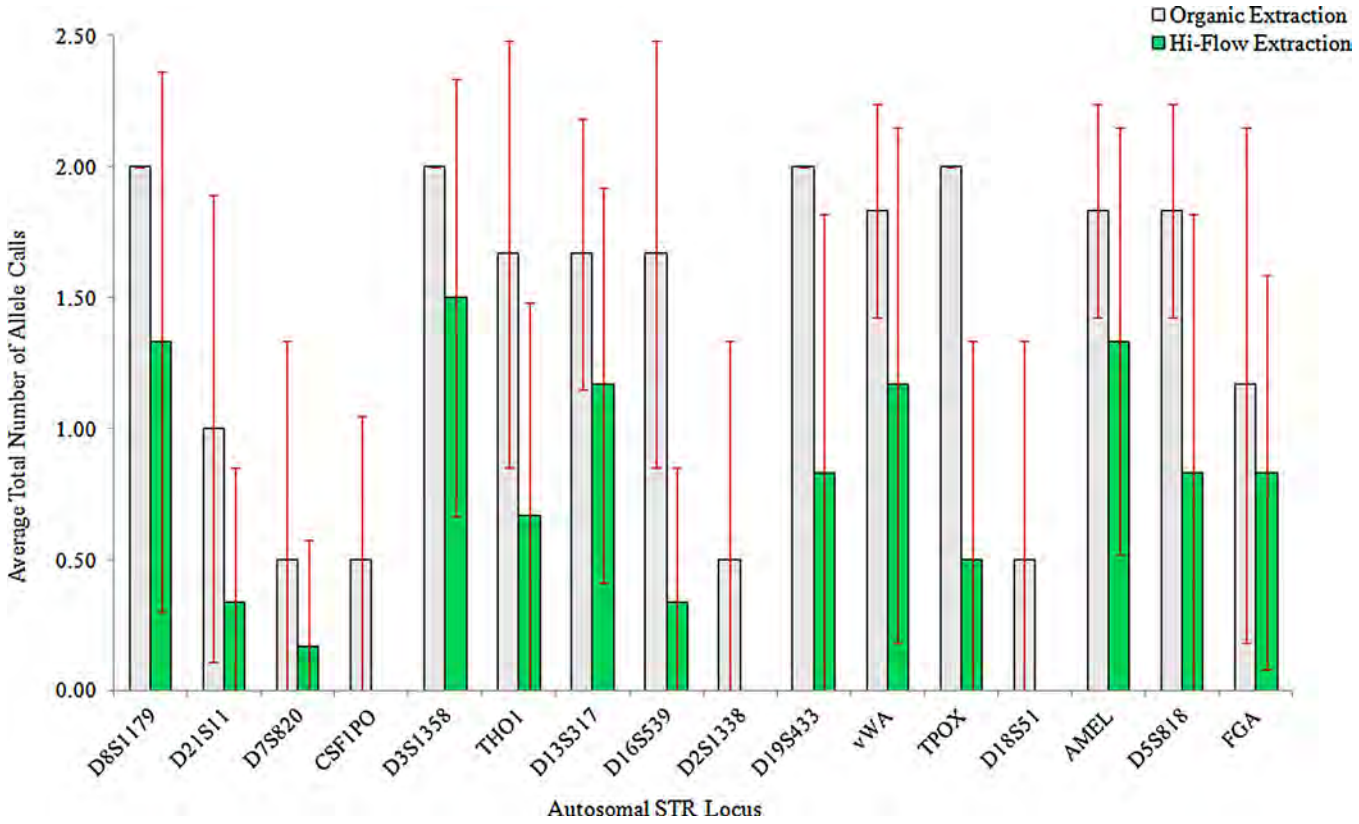

Fig. 2. Organic vs. Hi-Flow® DNA extraction: comparison of the average number of allele calls per autosomal STR locus after PCR amplification with AmpF*l*STR® Identifiler® Plus (analytical threshold = 75 RFU; n = 12 bone samples).

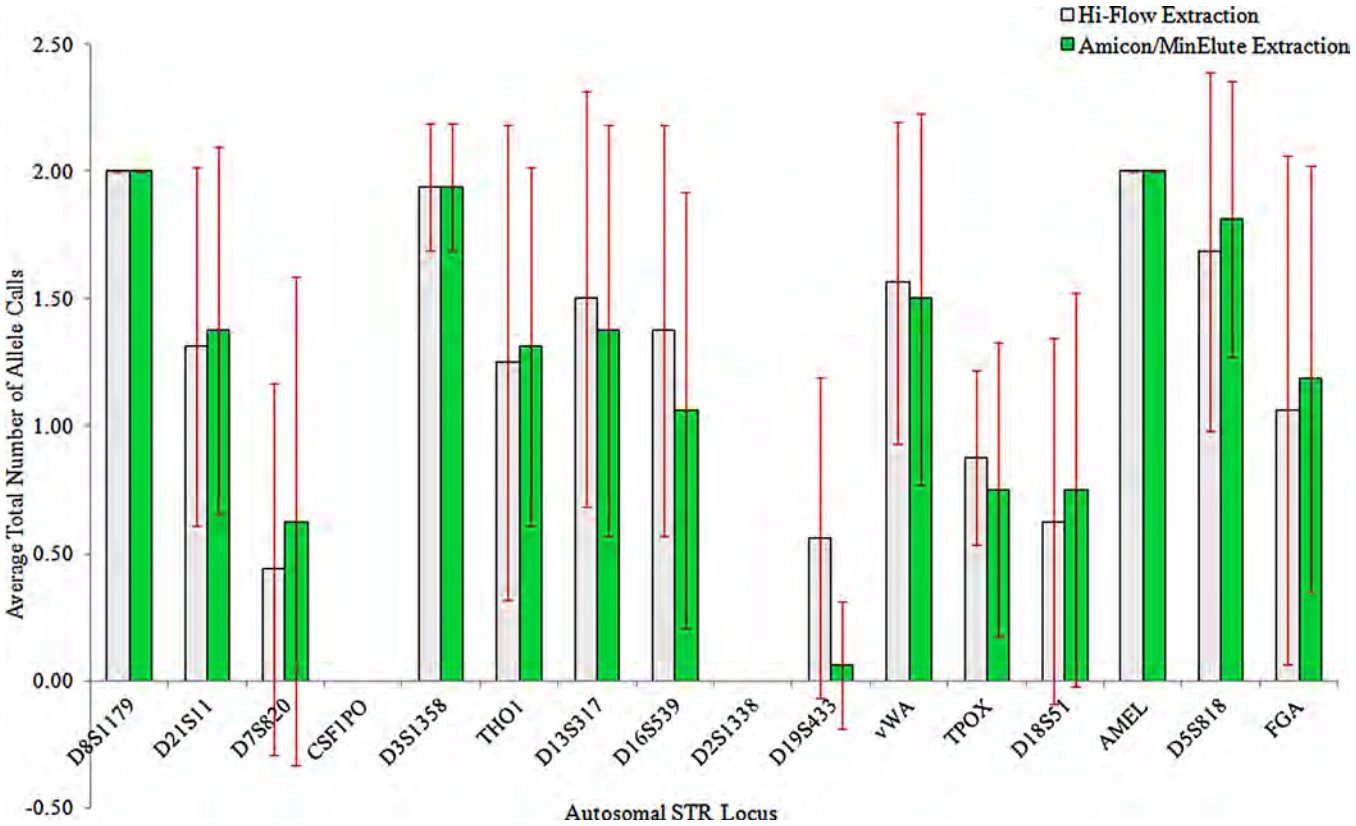

Fig. 3. Hi-Flow® vs. Amicon®/MinElute® DNA extraction: comparison of the average number of allele calls per autosomal STR locus after PCR amplification with AmpFlSTR® Identifiler® Plus (analytical threshold = 75 RFU; n = 32 bone samples).

locus for twelve bone samples (6 tibiae, 6 femora). Similar to that observed with autosomal DNA, the organic extraction method outperformed Hi-Flow® extraction for virtually all loci examined. This observation further suggests that using a column during DNA extraction (whether it functions in silica binding or via size exclusion) may not be as effective when working with low-quantity and/or low-quality DNA samples, presumably due to loss of DNA during column washing and/or retention of DNA in the column after elution. Similar conclusions were reported by Noren et al. [22]. Unlike the results from autosomal testing, the Amicon® Ultra-4/MinElute® DNA extraction method resulted in a higher average number of allele calls per locus when compared to the Hi-Flow® protocol (Fig. 5). A direct comparison between the organic and Amicon® Ultra-4/MinElute® DNA extraction methods could not be performed due to lack of sufficient bone powder fractions for testing.

3.3. Familial relationship testing

The principal goal of this project was to determine whether a familial link exists between Ezekiel Harper and his alleged son, Earl J. Maxwell. Since Earl J. Maxwell has not been exhumed, no direct samples were available for DNA testing. As shown in Fig. 6, Earl fathered seven children, only one of whom was still living at the time of this study. Samples from Earl's wife and his mother also were not available for testing, and 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. Of Earl's seven children, only two of them were male; the sons of these two males (i.e. Earl's grandsons) submitted buccal cell reference samples for comparison to Ezekiel's Y-STR haplotype (Fig. 6).

The Y-STR haplotypes obtained from both of Earl J. Maxwell's grandsons were identical to each other and to the alleles in Ezekiel Harper's consensus profile at all 17 loci examined. Two separate Y-STR haplotype reference databases were accessed for statistical analysis: the U.S. Y-STR Database (www.usystrdatabase.org) and YHRD (www.yhrd.org) [17]. The Y-STR haplotype obtained from the skeletal remains of Ezekiel Harper and from the two alleged grandsons' reference samples was not found in either database. Since Ezekiel Harper was known to be of European (German) descent, likelihood ratios (LRs) were calculated using sample sizes from similar affine populations. Table 3 summarizes the LR results when applying the 95% upper bound confidence interval.

Using Caucasian haplotypes from the U.S. Y-STR database, it is 1667 times more likely to observe the Y-STR results if Ezekiel Harper and Earl Maxwell are paternally related as opposed to being unrelated. With YHRD data for Western and Eastern European Y-STR haplotypes, the LRs are 2500 and 1111, respectively. Using the entire European metapopulation (combined Western and Eastern European), the LR is 3333.

3.4. DNA survival

The anthropologist who performed the exhumation of Ezekiel's remains collected soil samples during the excavation for pH testing (EPA Method 9045D). The soil was found to be rather acidic (pH range: 4.67–5.16 from three samples: control, topsoil, and "at-remains" level). Ezekiel's casket was buried approximately six feet (~2 m) beneath the soil surface and was constructed of wood (which was common in the 1800s). However, the casket was

Table 2
Comparison of allele calls for three bone and tooth extracts using two different Y-STR PCR amplification kits (PowerPlex® Y23 and AmpF*l*STR Yfiler®).

	DYS456	DYS389 I	DYS390	DYS389 II	DYS458	DYS19	DYS385 a/b	DYS393	DYS391	DYS439	DYS635	DYS392	Y GATA H4	DYS437	DYS438	DYS448	PCR amplification kit
Tibia 008.001_Hi-Flow-E1	15	13	24	29	16			13	11		23	13				19	PowerPlex® Y23
Tibia 008.001_Hi-Flow-E1								13	11		23			15		19	AmpF*l*STR Yfiler®
R.femur 001.002_Amicon-E1		13	24		16		11,16	13	11	11	23	13				19	PowerPlex® Y23
R.femur 001.002_Amicon-E1		13						13	11					15			AmpF*l*STR Yfiler®
L.lateral incisor_Amicon-E1		13			16		11,16	13	11		23					19	PowerPlex® Y23
L.lateral incisor_Amicon-E1	15							13	11				12				AmpF*l*STR Yfiler®

Table 3
Summary of statistical analyses from Y-STR population database searches for the haplotype obtained from both Ezekiel Harper's skeletal remains and the reference samples from alleged family members.

Population	Y-STR reference database	Total number of haplotypes in database	Frequency in database	Frequency upper bound (95% CI)	Likelihood ratio
U.S. Caucasian	www.usystrdatabase.org	6035	0	0.0006	1667
Western European	www.yhrd.org	8986	0	0.0004	2500
Eastern European	www.yhrd.org	3959	0	0.0009	1111
European metapopulation (combined)	www.yhrd.org	12,945	0	0.0003	3333

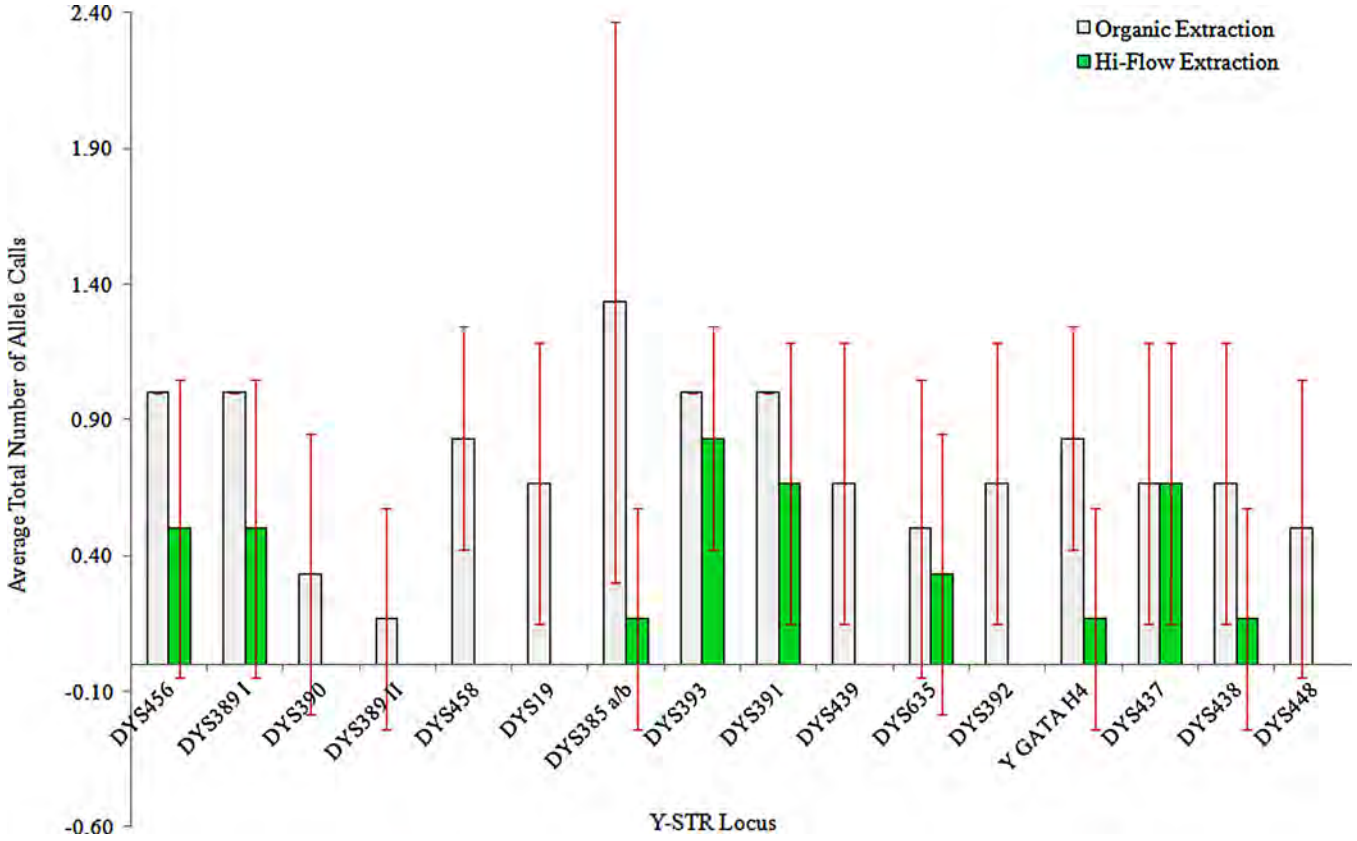

Fig. 4. Organic vs. Hi-Flow® DNA extraction: comparison of the average number of allele calls per Y-STR locus after PCR amplification with AmpF*l*STR Yfiler® (analytical threshold = 150 RFU; $n = 12$ bone samples).

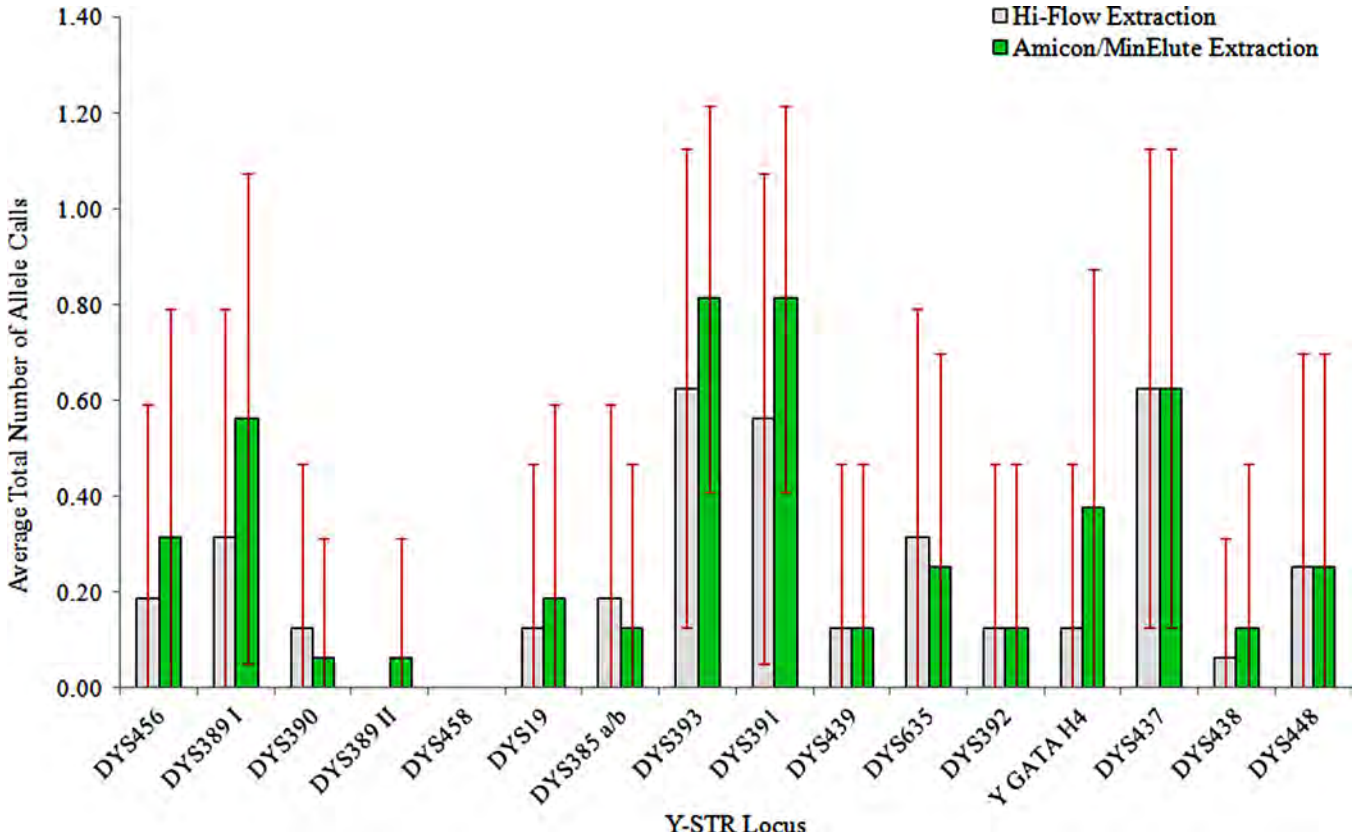

Fig. 5. Hi-Flow® vs. Amicon®/MinElute® DNA extraction: comparison of the average number of allele calls per Y-STR locus after PCR amplification with AmpF*l*STR Yfiler® (analytical threshold = 150 RFU; $n = 32$ bone samples).

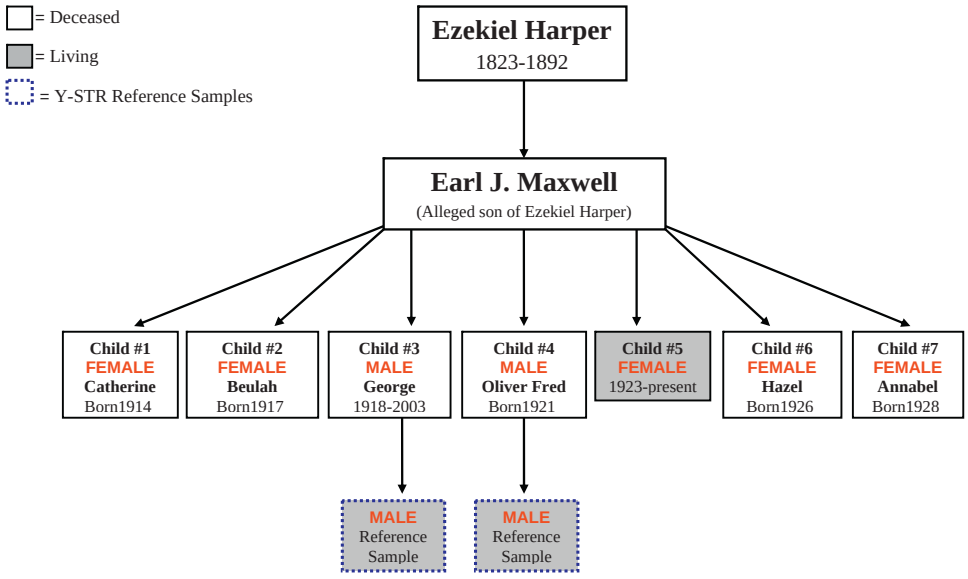

Fig. 6. Pedigree of the alleged familial association between Ezekiel Harper, Earl J. Maxwell, and Maxwell's descendants. Earl's seven children are listed in chronological birth order with dates of birth and death (if known). Two of Earl's grandsons submitted reference samples for this study.

unusual in the sense that it was lined entirely in high-quality glass. A local funeral director assisting in the exhumation noted that the quality of the glass used in the casket lining (i.e. pristine with no bubbles) would have been very expensive during that time period and was traditionally used only in caskets of wealthy, prominent citizens. Although it was discovered upon exhumation that the glass-lined wooden casket had collapsed under the weight of the soil, when this event occurred is unknown. Hence, Ezekiel's remains were protected by a double-layered physical barrier for at least a portion of the 120-year burial period, which in turn shielded him (and his DNA) from some environmental insults. Additionally, embalming was an uncommon practice in the region at the time of Ezekiel's death, and according to the anthropologist and funeral director who handled his remains, there was no indication that he had been embalmed. This lack of embalming coupled with the protection afforded by the construction of his casket present conditions that may explain the survival of Ezekiel's DNA more than a century after his burial.

4. Conclusions

Although the remains of Ezekiel Harper are more than a century old, consensus profiles of both autosomal and Y STRs were generated. As might be expected with old human remains, there was evidence of allele drop-out in all samples tested, and minor incidences of allele drop-in were observed in a few samples. Consensus and combined approaches were chosen to develop the reported profiles. The approach of combining all data to generate the resultant profiles is supported by the anthropological data of only one individual contributing to the remains and the single-source nature of the particular samples. The Y-STR profile was suitable for determining whether there is a potential kinship relationship between Ezekiel and Earl J. Maxwell via Maxwell's living male descendants. The Y-STR haplotypes obtained from both of Earl J. Maxwell's grandsons were identical to each other and to the alleles in Ezekiel Harper's consensus profile at all 17 loci examined. The Y-STR genetic evidence supports the hypothesis that Earl J. Maxwell is the son of Ezekiel Harper. Additionally, 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 the rarity of its occurrence and further strengthens this conclusion of kinship.

Acknowledgments

We would like to thank Dr. George Rhoades and the Maxwell family for providing photographs and historical information about Ezekiel Harper and Earl Maxwell; for their willingness to provide appropriate reference samples for comparison; and for their patience during the extensive time period that it took to complete this project. We deeply appreciate the assistance of Steve French, Civil War historian and author, for his help in gathering biographical records about Ezekiel Harper for this publication. We also would like to express our gratitude to the Lohr and Barb Funeral Home (Parsons, West Virginia) for copies of the exhumation photographs and for providing historical reference information about burials and casket construction in the late 19th century.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.fsigen.2013.10.014.

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