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# Mitochondrial DNA Analysis of the Presumptive Remains of Jesse James*

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**ABSTRACT:** We report here the results of mtDNA analysis of remains exhumed in July, 1995 from Mt. Olivet Cemetery in Kearney, Nebraska, that are thought to be those of Jesse James. The remains were poorly preserved, presumably due to wet and slightly acidic soil conditions, and insufficient DNA for analysis was obtained from two bone samples. However, two of four teeth, and two hairs recovered in 1978 from the original burial site on the James Farm, did yield reproducible mtDNA sequences. These mtDNA sequences from the teeth and hairs were all identical, suggesting that they came from the same individual; furthermore, this mtDNA sequence was identical to mtDNA sequences determined from blood samples from two maternal relatives of Jesse James. Therefore, either the remains are indeed those of Jesse James, or they are from an unrelated individual who, by chance, happens to have the same mtDNA sequence. To assess the probability that an unrelated individual would have the same sequence, we searched the forensic mtDNA database, and found that this sequence does not appear among the 2426 mtDNA sequences therein. Hence, the mtDNA analysis supports the identification of the exhumed remains from Mt. Olivet Cemetery as those of Jesse James.

**KEYWORDS:** forensic science, human identification, DNA typing, mitochondrial DNA, Jesse James, exhumation

Due to its high copy number, rapid rate of evolution, and haploid, maternal mode of inheritance, mitochondrial DNA (mtDNA) offers certain advantages over autosomal DNA markers for the identification of human remains (1). This is particularly true when the remains in question are of historical nature. The high copy number, with several hundred mtDNA molecules per cell (2), means that with older remains there is a greater likelihood of success in analyzing mtDNA as opposed to autosomal DNA, simply because mtDNA is so much more abundant to start with. The rapid rate of evolution means that there is a correspondingly high probability of exclusion if the remains are indeed not from the individual in question. Most importantly, for historical remains where the living relatives that are available as sources of reference DNA might be several generations removed from the individual in question, identification based on nuclear DNA is essentially impossible. This is because after several generations of segregation and recombination, very little nuclear DNA would be shared by the living relatives and the individual in question. However, the haploid and maternal mode of inheritance of mtDNA means that any living maternal relative of the individual in question should have an identical mtDNA type, and can thus serve as a reference source. In actual practice, mtDNA analysis was instrumental in identifying the putative remains of the Romanovs (3,4), as well as in determining the true identity of Anna Anderson (5).

The circumstances surrounding the death of the legendary outlaw Jesse James have also been in dispute. In 1882, James was reportedly living in St. Joseph under the name of Thomas Howard with his wife, Zerelda, and their two children. The conventional view is that Robert Ford, a fellow member of the James brothers' gang, shot him in the back of the head on April 3, 1882, killing him instantly, as he adjusted a picture on the wall of the living room at his home. However, some individuals claim that someone else died in his place, and that Jesse James actually survived to father additional children.

After his death, the remains of "Thomas Howard" were transported to the birthplace of Jesse James in Kearney, Missouri where they were buried on April 6, 1882 in the front yard of the family farm. This burial site was selected by the James family to prevent the possible theft or desecration of the remains. On June 29, 1902, the remains at the farm were removed, placed in a new casket, and reburied in the family plot at the Mt. Olivet Cemetery in Kearney.

In October 1978, the original grave at the James farm was excavated, and bone fragments, hair, and artifacts were recovered. The hair and the artifacts were retained for display at the James farm, then an historic site under the ownership of Clay County, Missouri, while the bone fragments were analyzed by Dr. Michael Finnegan of the Kansas State University and afterwards reburied at the original farm site. In 1995, a court order for the exhumation of the remains from the grave in the family plot at Mount Olivet Cemetery in Kearney was obtained by one of the authors (J. Starrs). The exhumation itself lasted several days (July 17–19) due to the decayed and fragmentary nature of the wooden casket and of the remains themselves.

Here, we report the results of mtDNA analysis of the remains exhumed from Mt. Olivet Cemetery in Kearney, Nebraska, that are thought to be those of Jesse James. MtDNA analysis was also per-

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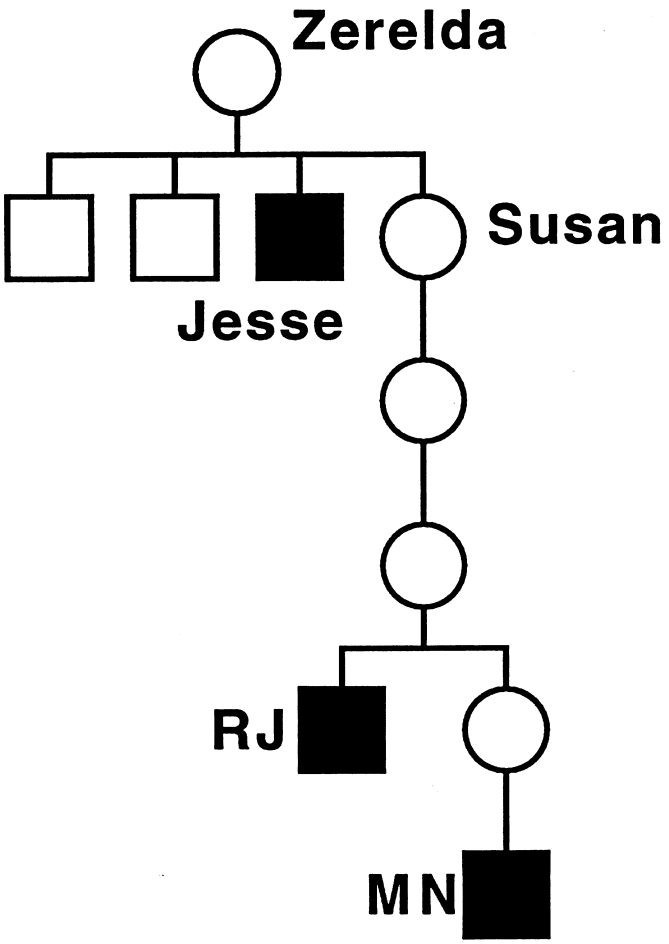


FIG. 1—*Geneology of the maternal relatives of Jesse James. Circles denote females and squares denote males. RJ is a great-grandson and MN is a great-great-grandson of Jesse's sister Susan, and thus both are expected to have the same mtDNA sequence as Jesse James.*

formed on hair recovered from the James farm following the excavation of the original grave site in 1978. Details concerning the exhumation procedure, as well as the genealogical analysis leading to the identification of the living maternal relatives of Jesse James, are available upon request from J. Starrs. For mtDNA analysis, direct descendants (either real or not) are of no use for identifying the presumptive remains, since Jesse James would not have transmitted his mtDNA to his descendants. Fortunately, Jesse James did have a sister, Susan, who would have had the same mtDNA type as Jesse, since both Susan and Jesse received their mtDNA from their mother, Zerelda. Two living maternal descendants of Susan (Fig. 1), her great-grandson (RJ) and her great-great-grandson (MN), were available and kindly consented to donate blood specimens to serve as maternal references for the mtDNA analysis of the exhumed remains. We report here the results of this analysis.

## Methods

Bone specimens, consisting of portions of the tibia and femur, and four teeth (three molars and a canine) were obtained for DNA analysis from the July, 1995 exhumation at Mt. Olivet Cemetery. Hair specimens were obtained from the original burial site at the James farm in 1978. To prevent contamination from prior handling, the outer layer of bone was removed with a rotary tool, while the teeth were briefly soaked in 10% bleach. The bone and teeth were then ground to a fine powder using a modified paint mixer with aluminum ceramic vials and ball bearings (SPEX Industries, NJ). DNA was isolated from the powdered bone and teeth, and from the hairs, by a silica-based extraction protocol (6). DNA was isolated from blood samples from two maternal relatives of Jesse James (MN and RJ, Fig. 1) with the use of the IsoQuick Kit (MicroProbe), according to the manufacturer's directions.

For the bone, teeth, and hair samples, a hot-start PCR (7) was used to amplify the first hypervariable segment (HV1) of the mtDNA control region in up to four overlapping fragments, as described previously (8,9). For the blood samples, the entire HV1 region was amplified via PCR with primers L15996 and H16401 (10). In each PCR, one primer was biotinylated, to facilitate the preparation of single-stranded DNA for sequencing by previously-described methods (11).

Extensive precautions were taken to avoid contamination of samples with extraneous DNA. All DNA extractions and preparation of PCR involving the exhumed remains were carried out in a laboratory physically separate from the laboratory in which PCR and post-PCR analysis was conducted. Disposable masks, gloves, and laboratory coats were worn throughout the above procedures and were changed frequently. The ceramic vials and ball bearings were rinsed with 10% bleach, followed by $ddH_2O$, and then UV-irradiated in between use. Dedicated reagents and pipettors were used, as were filter-plugged tips; pipettors were UV-irradiated in between use. All DNA extractions and PCR reactions included negative controls that contained all reagents except for tissue or DNA.

## Results and Discussion

The initial attempts at mtDNA analysis of the bone specimens from the exhumed remains were not successful (Table 1). No visible PCR product was obtained from the bone specimens despite numerous attempts, as judged by agarose gel electrophoresis followed by staining with ethidium bromide. The state of preservation of the bone samples was poor, probably reflecting the wet and slightly acidic soil conditions at the Mt. Olivet Cemetery, which thus probably accounts for the lack of DNA. In our forensic case work involving mtDNA analysis we have observed that DNA tends to preserve better in teeth than in bones (M. Stoneking and A. C. Stone, unpublished results); we therefore obtained four teeth for analysis.

One of the teeth (the canine, Table 1) still did not yield sufficient DNA for analysis. This tooth was not as well-preserved as the other teeth, as flaking of the tooth was observed during handling. MtDNA sequences were obtained from another tooth; however,

TABLE 1—*Summary of the results of DNA extractions from the exhumed remains.*

| Extract | Source | Result |
|---|---|---|
| A | Tibia | Insufficient DNA |
| B-1 | Femur | Insufficient DNA |
| B-2 | Femur | Insufficient DNA |
| B-3 | Femur | Insufficient DNA |
| C | Molar tooth | Successful |
| D | Canine tooth | Insufficient DNA |
| E | Molar tooth | Non-reproducible sequences |
| F | Molar tooth | Successful |
| H-1 | Hair | Successful |
| H-2 | Hair | Successful |

different sequences were obtained from the same DNA extract. Lack of reproducibility of DNA sequences from ancient specimens has been observed before (12), and has been attributed to contamination by extraneous DNA. Regardless of the actual cause, this lack of reproducibility means that none of the DNA sequences obtained from this extract can be used in the identification of the remains, as one of the scientific criterion for accepting the authenticity of an mtDNA sequence is that the same sequence must be obtained when the analysis is repeated (13).

Fortunately, reproducible mtDNA sequences were obtained from two molars (Table 1), with no contamination observed in the extraction or PCR blanks. Reproducible mtDNA sequences were obtained from two hairs as well (Table 1). A total of 302 nucleotides of mtDNA sequence were obtained, corresponding to positions 16055–16356 of the published reference sequence (14). The mtDNA sequences from the two teeth and from the two hairs were identical, differing from the published reference sequence (14) at five nucleotide positions (Fig. 2). Therefore, it is likely that the teeth and hairs came from the same individual.

MtDNA sequences were determined from blood samples of two maternal relatives of Jesse James, RJ and MN (Fig. 1). These two sequences were identical to each other, as expected if RJ and MN are maternally-related; they were also identical to the mtDNA sequence obtained from the teeth and hair (Fig. 2).

MtDNA analysis is, like any DNA analysis, a test for exclusion. If the mtDNA sequence of a case sample is different from a maternal reference mtDNA sequence, then the case sample cannot come from a maternal relative of the reference sample, and the theoretical probability of exclusion is 100% (in actual practice the probability of exclusion is less than 100%, since sample mixups, laboratory errors, or contamination with extraneous DNA can lead to false exclusions). In this particular case, the mtDNA sequences were identical, so the test for exclusion failed. We are left with three possibilities: (1) the exhumed remains are indeed those of Jesse James; (2) the exhumed remains are not Jesse James, but from another maternal relative of RJ and MN; or (3) the exhumed remains are from an unrelated individual who, by chance, happens to have the same mtDNA sequence as RJ and MN.

To assess the likelihood of this third possibility, we searched the forensic mtDNA database, maintained by the Technical Working Group on DNA Analysis Methods (TWGDAM), for additional occurrences of this same sequence. The mtDNA sequence we obtained from the exhumed remains, and from RJ and MN, was not found in the database of 2426 sequences (consisting of 1219 Europeans or European-Americans, 563 African-Americans, 342 Asians and 302 Hispanics). It therefore seems unlikely that an unrelated individual would have this same mtDNA sequence.

Do the mtDNA results prove that the exhumed remains are those of Jesse James? The answer to this question must be no, as there is always the possibility (however remote) that the remains are from a different maternal relative of RJ and MN, or from an unrelated person with the same mtDNA sequence. However, it should be emphasized that the mtDNA results are in complete agreement with the other scientific investigations of the exhumed remains: there is no scientific basis whatsoever for doubting that the exhumed remains are those of Jesse James. The burden of proof now shifts to those who, for whatever reason, choose to still doubt the identification. The mtDNA results reported herein provide a standard which other claimants to the legacy of Jesse James must satisfy.

| | Nucleotide Position | | | | |
|---|---|---|---|---|---|
| | 16126 | 16147 | 16223 | 16266 | 16304 |
| Sample | 6 | 4 | 4 | 6 | 4 |
| Reference | T | G | C | C | T |
| C | C | A | T | T | C |
| F | C | A | T | T | C |
| H-1 | C | A | T | T | C |
| H-2 | C | A | T | T | C |
| RJ | C | A | T | T | C |
| MN | C | A | T | T | C |

FIG. 2—*MtDNA sequences obtained from the exhumed teeth and hair, and from the maternal relatives of Jesse James. "Reference" is the published complete mtDNA sequence (13), and nucleotide positions are numbered according to the reference sequence; the mtDNA sequences from the remains (C, F, H-1, and H-2; Table 1) and from the maternal relatives (RJ and MN; Fig. 1) are shown as differences from the reference sequence.*

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