DNA Makes History

One night in July 1918, Tsar Nicholas II of Russia and his family met gruesome deaths at the hands of Bolsheviks in a Ural mountain town called Ekaterinburg. Captors led the tsar, tsarina, three of their daughters, the family physician, and three servants to a cellar and shot them, bayoneting those who did not immediately die. The executioners then stripped the bodies and loaded them onto a truck, planning to hurl them down a mine shaft. But the truck broke down, and the killers instead placed the bodies in a shallow grave, then damaged them with sulfuric acid to mask their identities.

In another July — many years later, in 1991 — two Russian amateur historians found the grave. Because they were aware that the royal family had spent its last night in Ekaterinburg, they alerted the government that they might have unearthed the long-sought bodies of the Romanov family. An official forensic examination soon determined that the skeletons represented nine individuals. The sizes of the skeletons indicated that three were children, and the porcelain, platinum, and gold in some of the teeth suggested royalty. Unfortunately, the acid had so destroyed the facial bones that some conventional forensic tests were not feasible. But one type of evidence survived — DNA. Thanks to PCR, researchers were able to obtain enough genetic material to solve the mystery.

British researchers eagerly examined DNA from cells in the skeletal remains. DNA sequences specific to the Y chromosome enabled the investigators to distinguish males from females. Then the genetic material of mitochondria, inherited from mothers only, established one woman as the mother of the children.

But a mother, her children, and companions do not make a royal family. The researchers had to connect the skeletons to known royalty. To do so, they again turned to DNA. However, an inherited quirk proved, at first, to be quite confusing.

The challenge in proving that the male remains with fancy dental work were once Tsar Nicholas II centered around nucleotide position 16169 of a mitochondrial gene whose sequence is highly variable among individuals. About 70 percent of the bone cells examined from the remains had cytosine (C) at this position, and the remainder had thymine (T). Skeptics at first suspected contamination or a laboratory error, but when the odd result was repeated, researchers realized that this historical case had revealed a genetic phenomenon called heteroplasmy. The bone cells apparently harbored two populations of mitochondria, one type with C at this position, the other with T.

The DNA of a living blood relative of the tsar, Countess Xenia Cheremeteff-Sfiri had only T at nucleotide site 16169. Xenia is the great-granddaughter of Tsar Nicholas II's sister. However, mitochondrial DNA from Xenia and the murdered man matched at every other site. DNA of another living relative, the Duke of Fife, the great-grandson of Nicholas's maternal aunt, matched Xenia at the famed 16169 site. A closer relative, Nicholas's nephew Tikhon Kulikovsky, refused to lend his DNA, citing anger at the British for not assisting the tsar's family during the Bolshevik revolution.

But the story wasn't over. It would take an event in yet another July, in 1994, to clarify matters.

Attention turned to Nicholas's brother, Grand Duke of Russia Georgij Romanov. In 1899, Gcorgij had died at age 28 of tuberculosis. He was exhumed in July 1994, and researchers sequenced the troublesome mitochondrial gene in bone cells from his leg. They found a match! Georgij's mitochondrial DNA had the same double-base site as the man murdered in Siberia, who was, therefore, Tsar Nicholas II. The researchers calculated the probability that the remains are truly those of the tsar, rather than resembling Georgij's unusual DNA sequence by chance, as 130 million to 1. The murdered Russian royal family can finally rest in peace, thanks to DNA analysis.