ANNALS OF HUMAN BIOLOGY
2004, 1–8, PREVIEW ARTICLE

Taylor&Francis
healthsciences

Molecular, forensic and haplotypic inconsistencies regarding the identity of the Ekaterinburg remains

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Received 10 September 2003; in revised form 30 October 2003; accepted 4 November 2003

Summary. *Background*: A set of human remains unearthed near Ekaterinburg, Russia has been attributed to the Romanov Imperial Family of Russia and their physician and servants. That conclusion was officially accepted by the Russian government following publication of DNA tests that were widely publicized. The published study included no discussion of major forensic discrepancies and the information regarding the burial site and remains included irregularities. Furthermore, its conclusion of Romanov identity was based on molecular behaviour that indicates contamination rather than endogenous DNA. The published claim to have amplified by PCR a 1223 bp region of degraded DNA in a single segment for nine individuals and then to have obtained sequence of PCR products derived from that segment without cloning indicates that the Ekaterinburg samples were contaminated with non-degraded, high molecular weight, 'fresh' DNA.
Aim: Noting major violations of standard forensic practices, factual inconsistencies, and molecular behaviours that invalidate the claimed identity, we attempted to replicate the findings of the original DNA study.
Subject: We analysed mtDNA extracted from a sample of the relic of Grand Duchess Elisabeth, sister of Empress Alexandra.
Results: Among clones of multiple PCR targets and products, we observed no complete mtDNA haplotype matching that reported for Alexandra. The consensus haplotype of Elisabeth differs from that reported for Alexandra at four sites.
Conclusion: Considering molecular and forensic inconsistencies, the identity of the Ekaterinburg remains has not been established. Our mtDNA haplotype results for Elisabeth provide yet another line of conflicting evidence regarding the identity of the Ekaterinburg remains.

1. Introduction

In 1918 the Family of Nicholas II, last Emperor of Russia, disappeared, presumably murdered by the Urals Reds. Shortly thereafter the White government appointed investigator, Judge N. Sokolov, concluded that The Family had been shot in Ekaterinburg, Russia, and that their bodies had been chopped into pieces, burned to shards of bone, and destroyed by soaking in sulphuric acid (Sokolov 1925, O'Conor 1971).

There are many remains buried in shallow, unmarked mass graves near Ekaterinburg, victims of the Russian Civil War (Summers and Mangold 1976). One such grave, assumed to contain remains of The Family, was claimed to be discovered and opened in 1991 (Gill *et al.* 1994). The case has been characterized by extreme irregularities at every level (*e.g.* Massie 1995, Zhivotovsky 1999,

Annals of Human Biology ISSN 0301–4460 print/ISSN 1464–5033 online © 2004 Taylor & Francis Ltd
http://www.tandf.co.uk/journals
DOI: 10.1080/03014460310001652257

McNeal 2001). Activities at the burial site and with the remains are no exception. Crucial evidence has been proven fraudulent. There is evidence that the 'Yurovsky Note', detailing the location of the grave and thereby providing the means for the 'discovery', was forged (Buranov 1994). The grave was of unknown age and had been opened several times prior to its official 'discovery' (Zhivotovsky 1999). The two 'discoverers' of the grave were described in Gill *et al.* (1994) as 'amateur historians' when in fact at least one was an agent of the Internal Minister of the USSR. They had opened the site in 1979 and removed several skulls and other bones and then added skulls and bones about a year later (Zhivotovsky 1999). There are also indications that the grave had been opened by the State Security services in 1946 (Koltypin-Wallovskoy *et al.* 1998). The purported site of the murder, the Ipatiev house in Ekaterinburg, was levelled and entirely destroyed in 1977 on the insistence of KGB Chairman Yuri Andropov and under the direction of Boris Yeltsin, at that time the First Secretary of the Sveldlovsk (Ekaterinburg) district branch of the USSR Communist Party (Massie 1995, McNeal 2001).

The Russian government has officially declared the case of identity of the remains purported to be those of the Romanov Imperial Family of Russia to be settled. Essential to that declaration was the report describing DNA results (Gill *et al.* 1994). At a conference in 1992 Russian geneticist P. Ivanov announced that he would conduct DNA analysis of the remains (Ivanov 1994). In 1994, the results of those DNA analyses were published, suggesting identity of Nicholas and Alexandra, three of their children, the family physician, and servants (Gill *et al.* 1994). That report did not mention numerous irregularities of the case. The summary of the events described in Gill *et al.* (1994) states that the grave was discovered in 1991 and tacitly introduces the assumption that its contents were intact. As detailed above, the remains were not intact at all. The chain of custody of the samples utilized in Gill *et al.* (1994) was not provided in the published report and has not been provided since. Furthermore, the authors have refused to provide their original 'raw' data to other scientists (McNeal 2001), and improper procedures by any individual who had control or access to the samples could have led to contamination and misidentification. The handling of the samples throughout the entire investigation has been characterized as 'rude violations of archaeological and forensic norms' (Krukov 1994). The statistical analyses of the DNA results were flawed (Zhivotovsky 1999). Individual bones rather than skeletons were the units of analysis (Zhivotovsky 1999), identity of individuals was disputed and the skeletal assignment of some bones was controversial (Massie 1995). The remains were in fragmented, deteriorated condition (Gill *et al.* 1994, Massie 1995). The skull attributed to the Emperor had no indication of the known scar from a sword wound acquired during an assassination attempt (McNeal 2001). The Russian co-author of the DNA report, P. Ivanov, had custody of all the bone samples from the remains, participated in the analyses, interpreted and summarized the findings on behalf of the Russian Governmental Commission for Study of Problems Related to Investigation and Reburial of the Remains of The Russian Emperor Nicholas II and the Members of his Family, and then voted on acceptance of the final communiqué (Russian Governmental Commission 1998, Zhivotovsky 1999). Note that the lengthy full name of the Russian Governmental Commission reveals an *a priori* bias toward the Commission's final conclusion.

During the last decade, the field of analysis of degraded DNA samples has progressed and much new technical knowledge has been gained. As the field has matured, rigorous

standards for peer review and publication have been established (Cooper and Poinar 2000, Hofreiter *et al*. 2001). Those standards were not in place at the time that the report on the DNA identity of the Ekaterinburg remains was reviewed for publication. If present knowledge had been available for referees at that time, the DNA evidence would have been judged in a qualitatively different context.

DNA extracted from deteriorated bones having been exposed in a shallow, damp, earthen mass grave for 70 years is chemically degraded (if detectable at all) and should behave as degraded DNA (*e.g*. Cooper and Poinar 2000, Hofreiter *et al*. 2001, Jehaes *et al*. 2001a, Stone *et al*. 2001). Fragments are sheared to smaller than 250 bp or are completely destroyed (e.g. Bailey *et al*. 1996, Jehaes *et al*. 2001b, Stone *et al*. 2001). Polymerase chain reaction (PCR) amplification of targets as large as 1223 bp, reported in Gill *et al*. (1994) for nine different individuals, is now regarded as certain evidence of contamination and invalidates results (Cooper and Poinar 2000, Hofreiter *et al*. 2001). PCR products of degraded DNA larger than 250 bp yield erroneous molecular results, whereas shorter products generally yield authentic results (Jehaes *et al*. 2001a). Yet, Gill *et al*. (1994) report that 'the quality of the sequence was generally comparable to that produced from the fresh blood samples'. DNA degrades rapidly after the death of an organism. Long before 70 years have passed, degradation reaches the 'ancient' condition (Jehaes *et al*. 2001a). Mitochondrial sequences must be amplified in short fragments (*e.g*. Cooper and Poinar 2000, Hofreiter *et al*. 2001, Jehaes *et al*. 2001b). Examples of similar historical cases, such as the identification of Louis XVII (Jehaes *et al*. 2001b) and Jesse James (Stone *et al*. 2001) serve to illustrate expected molecular behaviours. Molecular behaviours reported in Gill *et al*. (1994) were wholly inconsistent with the behaviours of degraded DNA and such behaviours have not been reported elsewhere for similar cases. That observation indicates that the samples assumed to be of the Romanovs were contaminated with non-degraded, high molecular weight, 'fresh' DNA.

A central tenet of science holds that results reported by a single research group do not establish conclusions with certainty. Noting the plethora of major problems associated with the entire Romanov investigation, we attempted to replicate the findings of the DNA study. In doing so, we uncovered evidence that casts yet additional doubt on the findings reported in Gill *et al*. (1994). In cooperation with the Russian Expert Commission Abroad, we have conducted mtDNA analysis of the relic of Grand Duchess Elisabeth Feodorovna, sister of Empress Alexandra. Bishop Anthony Grabbe of the Russian Orthodox Church Abroad, who oversaw the opening of Elisabeth's coffin in Jerusalem in 1981 and has kept the relic since that time, allowed collection of the sample. Controlled experiments of the molecular behaviour of the relic DNA were consistent with all other such old samples analysed in the literature, in that PCR amplification was not attainable for fragments larger than 250 bp. Two independent tests were performed, one at Stanford University and the other at Los Alamos National Laboratory.

2. Materials and methods

2.1. Chain of custody

The chain of custody of the remains of Grand Duchess Elisabeth is as follows (Sokolov 1925, Koehler 1988, Millar 1991). There is historical information from local people that Elisabeth remained alive for some time in a mineshaft near Alapaevsk after grenades were tossed by the Red executioners. Locals for fear of the Reds did not rescue her. Her body was identified by Elisabeth's Father

Confessor, Monk Seraphim, and by the White government commission headed by N. Sokolov. Sokolov described the body:

> On the chest of Grand Duchess Elisabeth Feodorovna there was an icon of our Saviour adorned with precious stones. According to my information, this was the same icon the Emperor prayed to before his abdication from the throne. He gave it to Elisabeth Feodorovna.

The body was washed, dressed, and placed in a wooden coffin. A memorial service was held on 18 October 1918, attended by 13 priests and many other people. On the morning of 19 October, the coffin was placed in a crypt beneath the Holy Trinity Cathedral. When the Red Army once more advanced on Alapaevsk, the coffin was shipped to eastern Siberia, then Shanghai, and from there to Jerusalem. On 15 January 1920, the coffin was met by English authorities, Greek and Russian clergy, and crowds of the Russian colony and locals. The following day the coffin was placed in a locked crypt beneath the St Mary Magdalene Russian Church where it remained for the next 62 years. From the time of the identification and sealing of the coffin, to Siberia, to Shanghai, and to interment in Jerusalem, Elisabeth's Father Confessor, Monk Seraphim, accompanied the coffin. On 1 May 1982, the coffin was opened. The ceremony was attended by Orthodox and non-Orthodox lay people, and clergy from many places including the USA, Australia, New Zealand and Europe. There was an inscription on the coffin of Elisabeth. It read, 'The body of the Grand Duchess Elisabeth Feodorovna, murdered by the bandits of the Soviet regime. Buried on 19 October 1918'. The relic was taken to New York by Bishop Anthony Grabbe, and there the sample was retrieved by one of us (A.K.) in the presence of the Bishop, who had attended the opening of the coffin and brought the relic from Jerusalem to New York. The relic has been kept in a small case in his reliquary.

2.2. *Sample preparation*

The relic of Grand Duchess Elisabeth provided by Bishop Grabbe is a finger resting in a small closed wooden case with a glass lid. The finger consists of bone and hardened, dried flesh. The case was opened to access the relic. With latex-gloved hands, a new, sterile scalpel was removed from its sealed package. Four small pieces of dried flesh and bone were cut and removed and placed with the scalpel blade into DNA-free microcentrifuge tubes, which were immediately sealed. Two of these tubes were opened and processed at Stanford University, and two others were opened and processed at Los Alamos National Laboratory.

2.3. *DNA extraction*

Each portion of the relic was digested in the original tube at 37°C for 72 h in a 500 μL solution of STE (100 mM $NaCl_2$, 10 mM Tris, 1 mM EDTA, pH 7.5) containing 20 μL 1 M dithiothreitol in 10 mM sodium acetate, pH 5.2; and 25 μL of 20% SDS. Fifty μL of 20 mg/mL^{-1} proteinase K in STE was added several times during the 3-day digestion, and the solution was mixed each time. The sample was partly, but not completely, digested by this process. Following digestion, DNA was isolated by two extractions in PCI (25:24:1 mixture of phenol, chloroform, and isoamyl alcohol, pH 8.0). Phenol was removed by two extractions with chloroform. The supernatant was collected, DNA was precipitated in ethanol and sodium acetate, pelleted at 10 000 × *g* for 10 min, dried, and resuspended in 0.1 × TE (1 mM Tris, 0.1 mM EDTA, pH 8.0).

2.4. PCR amplification

PCR experiments were conducted to test the molecular behaviour of the relic DNA. Five different PCR reactions were performed, each with a positive control consisting of high molecular weight DNA from a human cell line, the relic DNA, and a negative control to test for contamination. The cell line was from an individual of East Africa of mitochondrial haplogroup L2, with an HV1 sequence different from that found for the relic of Grand Duchess Elisabeth. In all cases, primers targeted mitochondrial regions. Target sizes were: 1179 bp, 466 bp, 437 bp, 128 bp and 108 bp. In all cases very strong amplification was observed for cell line DNA. All negative controls were blank. For the relic, no amplification occurred for 1179, 466 or 437 bp targets. Weak amplification occurred for the 128 bp target. Moderate amplification occurred for the 108 bp target. PCR primers for the 108 bp target were L16060 (AGATTTGGGTACCACCCAAG) and H16168 (GGGGTTTTGATGTGGATTGG). To test the region spanning the position 16357, a 111 bp target was amplified. PCR primers for the 111 bp target were L16273 (AGCCACCCCTCACCCACTAG) and H16384 (GTGGTCAAGGGACCCCTATCTG). Those primers produced strong amplification of the relic DNA.

Several PCR amplifications of the relic DNA were conducted separately from positive controls. Reactions were prepared on ice, and placed directly into an Applied Biosystems 9700 or MJ Research PTC-200 thermal cycler preheated to 94°C. Touchdown PCR was employed. Denaturing was at 94°C for 15 s. Annealing was initially at 65°C, dropping 0.5°C each cycle for 20 cycles to 55°C, followed by 20 cycles at 55°C. Annealing time was 30 s. Extension was at 72°C for 30 s. Following amplification, a single 2-min extension step at 72°C was employed.

2.5. Cloning of PCR products

Direct cloning of PCR products was performed by TA cloning into the pGEMT-Easy vector (Promega, Madison, WI, USA), followed by transformation in competent *E. coli* TOP10F′ cells (Invitrogen Life Technologies, Carlsbad, CA, USA). Plasmid DNA was isolated using either the Concert Rapid Plasmid Miniprep System (Invitrogen Life Technologies) or the Wizard Plus SV Minipreps DNA Purification System (Promega) and analysed by *Eco*RI digests, agarose gel electrophoresis, and sequenced.

2.6. Sequencing

PCR products were prepared for sequencing using QIAGEN (Valencia, CA, USA) QIAquick PCR Purification columns. PCR products and plasmids were sequenced using Applied Biosystems (Foster City, CA, USA) BigDye Terminator Cycle Sequencing Ready Reaction DNA Sequencing Kit, following the manufacturer's instructions. Ready Reaction premix was diluted four-fold using Applied Biosystems 5X sequencing buffer. PCR primers were used for direct sequencing. T7 (TAATACGACTCACTATAGGG) and SP6 (ATTTAGGTGACACTATAG) primers were used to sequence clones. Sequences were separated and visualized on Applied Biosystems genetic analysers.

The sample was collected by A.K. DNA extractions, PCRs, and sequencing were carried out separately by A.K. (at Stanford) and L.D.G. (at Los Alamos). TA cloning of PCR products was carried out by D.H.K. at Eastern Michigan University. Sequences of mtDNA of A.K. and L.D.G. differ from the sequence found for Elisabeth and for the purported Alexandra.

3. Results

3.1. Direct sequencing

Direct sequencing of PCR products of Elisabeth's mtDNA revealed a haplotype different than that reported in Gill *et al.* (1994) for the remains of the purported Alexandra. The sequence of purported Alexandra had two differences in hypervariable region 1 in comparison to the reference sequence (Anderson *et al.* 1981), 16111T and 16357C. It is notable that this haplotype has not been reported elsewhere. Between PCR primers L16060 and H16168, direct sequencing revealed a haplotype for Elisabeth that differs from that of purported Alexandra at two sites, having 16111C and 16129A. Between PCR primers L16273 and H16384, direct sequencing revealed a haplotype for Elisabeth that differs from that of purported Alexandra at two additional sites, having 16327T and 16357T. Sisters are expected to have identical mtDNA haplotypes.

3.2. Cloning

We performed two additional PCR amplifications of each region, of which direct sequencing produced results identical to the first tests. We then cloned these four PCR products and sequenced the clones, remaining consistent with the newly established guidelines (Cooper and Poinar 2000, Hofreiter *et al.* 2001) for degraded DNA. Of 19 clones of products of L16060-H16168 (table 1), two clones matched the partial haplotype of purported Alexandra, having 16111T and 16129G. The haplotype 16111T with 16357C, reported in Gill *et al.* (1994) for Alexandra, is otherwise unknown and was not observed in over 17 000 mostly European mtDNAs (unpublished database of Estonian Biocentre, Tartu) or in the extensive European data set of Richards *et al.* (2000). Seventeen clones matched the direct sequence result for Elisabeth. For the products of L16273-H16384, of 21 clones, none matched purported Alexandra, 16 matched the direct sequence result for Elisabeth, and five mismatched both (table 2). We call attention to the fact that such a level of multiple sequences is typical in clones of ancient DNA (aDNA) PCR products from truly ancient samples and more recent forensic samples (e.g. Krings *et al.* 1997, Jehaes *et al.* 2001b).

4. Discussion

Standing alone, the molecular behaviours reported in Gill *et al.* (1994) negate the conclusion of that report. That single factor is the basis for our conclusion that the samples analysed in the study of Gill *et al.* (1994) were contaminated. Our doubts of the identity are further supported by the context of gross violations of forensic

Table 1. Nucleotides at positions 16111 and 16129 in clones of PCR products E1 and E2, produced by PCR amplification with primers L16060 and H16168. No asterisk indicates match to direct sequence of Elisabeth PCR product.

PCR product	No. of clones	Haplotype
E1	9	16111C, 16129A
	1	16111T, 16129G*
E2	8	16111C, 16129A
	1	16111T, 16129G*

* Match to purported Alexandra.

Table 2. Nucleotides at positions 16327 and 16357 in clones of PCR products E3 and E4, produced by PCR amplification with primers L16273 and 16384. No asterisk indicates match to direct sequence of Elisabeth PCR product.

PCR product	No. of clones	Haplotype
E3	8	16327T, 16357T
	2	16327C, 16357T*
E4	8	16327T, 16357T
	3	16327C, 16357T*

*Mismatch to both purported Alexandra and Elisabeth.

investigative norms and factual inconsistencies. In light of those considerations, possible explanations for our results include the following.

(1) The actual sequence of Elisabeth does not match the sequence reported in Gill *et al.* (1994). In that case it is probable that the Ekaterinburg remains were misidentified, as the identity and chain of custody of the remains of Elisabeth are well known and documented (Sokolov 1925, Koehler 1988, Millar 1991).
(2) The actual sequence of Elisabeth does match the sequence reported in Gill *et al.* (1994). In that case, the endogenous actual sequence of the relic of Elisabeth is in extremely low copy number and old, degraded contamination is in relatively higher copy number. Therefore, the endogenous sequence was not observed among 21 clones of the L16273-H16384 region and only two of 19 clones of the L16060-H16129 region. In that case, as indicated by the molecular behaviours reported in Gill *et al.* (1994), the samples from the Ekaterinburg remains were contaminated in the study of Gill *et al.* (1994) with non-degraded, high molecular weight DNA from a source of the same mtDNA lineage as carried by Elisabeth.

Regardless of the explanation for our results, the molecular behaviours in Gill *et al.* (1994) leave the DNA identity of the Ekaterinburg remains as an open question. Standard practice is to establish endogenous sequence of degraded DNA by consensus of clones. The mismatch of the consensus sequence of Elisabeth, and the lack of the clones of the two regions analysed in combination to produce the haplotype attributed to Empress Alexandra, is simply yet another discrepancy in this unresolved case.

Acknowledgements

We thank Bishop Anthony Grabbe for allowing the test of the relic of Elisabeth. We thank Peter N. Koltypin-Wallovskoy and Eugene L. Magerovsky of the Russian Expert Commission Abroad for their support in many ways. We thank Michelle Reid of QIAGEN, and Peter A. Underhill, for technical advice. We thank NIH (GM2842 to J.L.M.) for partial funding.

This study was partly funded by NIH GM28428 to J.L.M.

References

Anderson, S., Bankier, A. T., Barrell, B. G., de Bruijn, M. H. L., Coulson, A. R., Drouin, J., Eperon, I. C., Nierlich, D. P., Roe, B. A., Sanger, F., Schreier, P. H., Smith, A. J. H.,

Staden, R., and Young, I. G., 1981, Sequence and organization of the human mitochondrial genome. *Nature*, **290,** 457–465.

Bailey, J. F., Richards, M. B., Macaulay, V. A., Colson, I. B., James, I. T., Bradley, D. G., Hedges, R. E. M., and Sykes, B. C., 1996, Ancient DNA suggests a recent expansion of European cattle from a diverse wild progenitor species. *Proc. R. Soc. Lond. B*, **263,** 1467–1473.

Buranov, Y. A., 1994, On the problem of the different versions and historical validity regarding the Ekaterinburg tragedy. In *Ekaterinburgskaya Tragediya*: *Taina Tsarskih Ostankov* [*The Ekaterinburg Tragedy*: *a Mystery of Tsar's Remains*]. (The Association of the Ural Publishers, Sredneural'skoe Knizhnoe Izdatel'stvo. Ekaterinburg), pp. 20–23 [in Russian].

Cooper, A., and Poinar, H., 2000, Ancient DNA: do it right or not at all. *Science*, **289,** 1139.

Gill, P., Ivanov, P. L., Kimpton, C., Piercy, R., Benson, N., Tully, G., Evett, I., Hagelberg, E., and Sullivan, K., 1994, Identification of the remains of the Romanov family by DNA analysis. *Nature Genetics*, **6,** 130–135.

Hofreiter, M., Serre, D., Poinar, H. N., Kuch, M., and Pääbo, S., 2001, Ancient DNA. *Nature Reviews Genetics*, **2,** 353–359.

Ivanov, P. L., 1994, Prospects of genetic analysis. In *Ekaterinburgskaya Tragediya*: *Taina Tsarskih Ostankov* [*The Ekaterinburg Tragedy*: *a Mystery of Tsar's Remains*]. (The Association of the Ural Publishers, Sredneural'skoe Knizhnoe Izdatel'stvo. Ekaterinburg), pp. 100–103 [in Russian].

Jehaes, E., Toprak, K., Vanderheyden, N., Pfeiffer, H., Cassiman, J.-J., Brinkmann, B., and DeCorte, R., 2001a, Pitfalls in the analysis of mitochondrial DNA from ancient specimens and the consequences for forensic DNA analysis: the historical case of the putative heart of Louis XVII. *International Journal of Legal Medicine*, **115,** 135–141.

Jehaes, E., Pfeiffer, H., Toprak, K., DeCorte, R., Brinkmann, B., and Cassiman, J.-J., 2001b, Mitochondrial DNA analysis of the putative heart of Louis XVII, son of Louis XVI and Marie-Antoinette. *European Journal of Human Genetics*, **9,** 185–190.

Koehler, L., 1998, *Saint Elisabeth The New Martyr* (New York: The Orthodox Palestine Society, U.S.A., Inc.).

Koltypin-Wallovskoy, P. N., Magerovsky, E. L., and Scherbatow, A. P., 1998, Memorandum No. 3 (open letter to the President of Russia). The Russian Expert Commission Abroad, Stratford, Connecticut, USA.

Krings, M., Stone, A., Schmitz, R. W., 1997, Neanderthal DNA sequences and the origin of modern humans. *Cell*, **90,** 19–30.

Krukov, V. N., 1994, The characteristics of mechanical damage to the bones and skeletons. In *Ekaterinburgskaya Tragediya*: *Taina Tsarskih Ostankov* [*The Ekaterinburg Tragedy*: *a Mystery of Tsar's Remains*], edited by N. E. Chuprjakova. (The Association of the Ural Publishers, Sredneural'skoe Knizhnoe Izdatel'stvo. Ekaterinburg), pp. 92–94 [in Russian].

Massie, R. K., 1995, *The Romanovs*: *The Final Chapter* (New York: Random House).

McNeal, S., 2001, *The Secret Plot to Save the Tsar* (New York: HarperCollins).

Millar, L., 1991, *Grand Duchess Elisabeth of Russia* (Redding, CA: Nikodemos Orthodox Publication Society).

O'Conor, J. F., 1971, *The Sokolov Investigation* (New York: Speller and Sons).

Richards, M., Macaulay, V., Hickey, E., Vega, E., Sykes, B., Guida, V., Rengo, C., Sellitto, D., Cruciani, F., Kivisild, T., Villems, R., Thomas, M., Rychkov, S., Rychkov, O., Rychkov, Y., Gölge, M., Dimitrov, D., Hill, E., Bradley, D., Romano, V., Calì, F., Vona, G., Demaine, A., Papiha, S., Triantaphyllidis, C., Stefanescu, G., Hatina, J., Belledi, M., Di Rienzo, A., Novelletto, A., Oppenheim, A., Nørby, S., Al-Zaheri, N., Santachiara-Benerecetti, S., Scozzari, R., Torroni, A., and Bandelt, H-J., 2000, Tracing European founder lineages in the Near Eastern mtDNA pool. *American Journal of Human Genetics*, **67,** 1251–1276. Available at: http://www.stats.ox.ac.uk/~macaulay/founder2000/index.html.

Russian Governmental Commission, 1998, *Materialy Pravitelstvennoy Komissii. Protokol #9* [The documents of the Russian Governmental Commission]. Available at: http://romanovy.narod.ru/protocol.htm, and http://romanovy.narod.ru/protocol.htm%20%5Bin%20Russian%5D.

Sokolov, N., 1925, *Ubiystvo Tsarskoy Sem'i* [*The Execution of the Tsar's Family*] (Slovo: Berlin) [in Russian]; 2nd edn, 1978 (Buenos Aires: El Verbo Ruso) [in Russian].

Stone, A. C., Starrs, J. E., and Stoneking, M., 2001, Mitochondrial DNA analysis of the presumptive remains of Jesse James. *Journal of Forensic Science*, **46,** 173–176.

Summers, A., and Mangold, T., 1976, *The File on the Tsar* (London: Victor Gollancz).

Zhivotovsky, L. A., 1999, Recognition of the remains of Tsar Nicholas II and his family: a case of premature identification? *Annals of Human Biology*, **26,** 569–577.

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