

Internet Resources

You may use other Internet sources here, but please cite any sources that you use unless they are one of the following.

1. <http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0004838> (You should be able to access the entire article. You may need to copy and paste the site address.)
2. <https://lastfiacorun.com/australia/who-took-over-after-czar-nicholas-ii.html>
3. <https://alphahistory.com/russianrevolution/white-armies/>
4. <https://www.royal.uk/prince-albert>
5. <https://www.genome.gov/Genetic-Disorders/Hemophilia>
6. <https://www.genome.gov/genetics-glossary/Carrier>
7. <https://www.nursingtimes.net/clinical-archive/haematology/haemophilia-pathophysiology-and-management-14-10-2003/>
8. <http://www.ncbi.nlm.nih.gov/pubmed/20557352> (You won't be able to access the entire article, but the abstract will give you important information.)
9. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5054066/>
10. <https://www.history.com/this-day-in-history/anastasia-arrives-in-the-united-states#:~:text=In%201968%2C%20Anne%20Anderson%20married,final%20years%20in%20Charlottesville%2C%20Virginia.>
11. <http://www.nature.com.proxy.lib.odu.edu/ng/journal/v9/n1/pdf/ng0195-9.pdf> (Please note that this is a PDF of an article.)
12. <https://medical-dictionary.thefreedictionary.com/hypervariable+region>
- 13.

History

1. Nicholas II was the last Romanov to hold power in Russia. What was his title?

Nicolas II has the title of the last ruling Russian Tsar.¹

2. How long had the Romanov family been in power in Russia?

The Romanov family was in power for over 300 years.¹

3. Nicholas II abdicated the throne. Who took power then?

“The Russian Provisional Government was a provisional government of the Russian Republic established immediately following the abdication of Tsar Nicholas II of the Russian Empire on March 2, 1917”.²

4. What happened to Nicholas II and his family after he abdicated the throne?

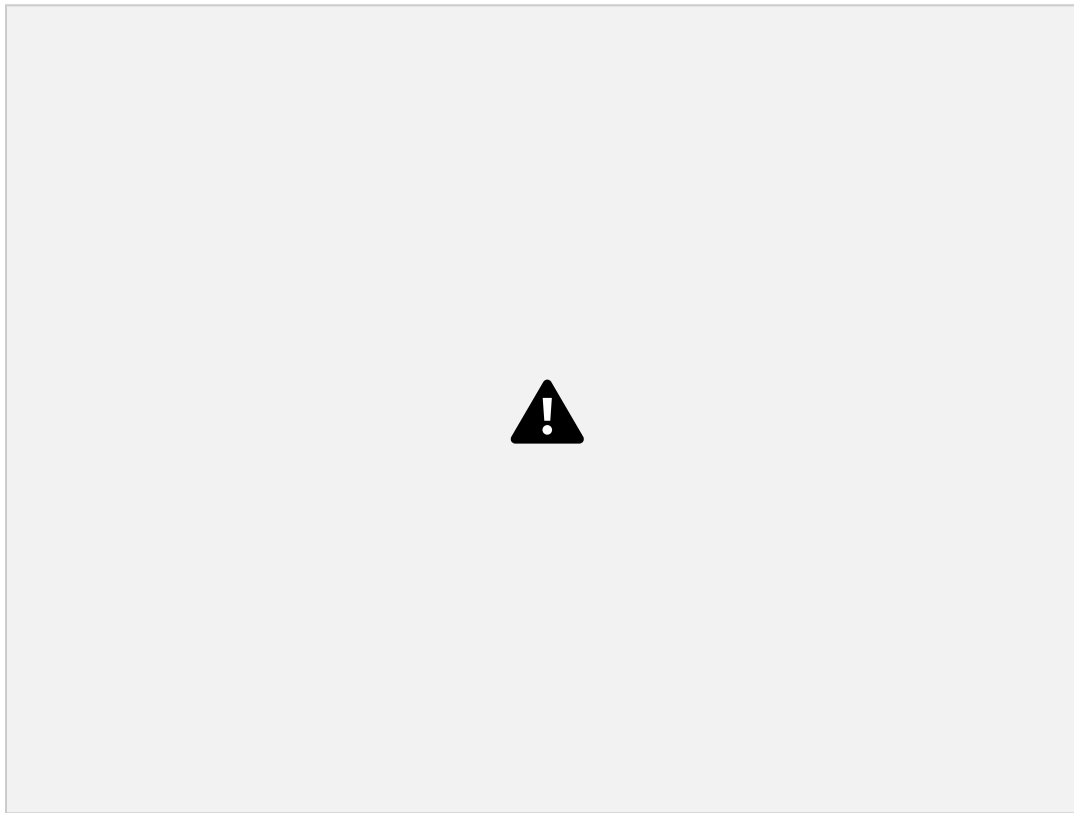
In July 17, 1918, the royal family and their staff were executed.¹

5. One of the reasons that the family of Nicholas II was executed (vs. just imprisoned) was because there was a fear that the White Russian Army would save them. Who was the White Russian Army?

“The White armies (also known as the ‘White Guards’ or ‘Whites’) were counter-revolutionary groups that participated in the Russian Civil War. These White armies fought against the Bolshevik Red Army for control of Russia.”³

Hemophilia

One of the pedigree charts found at the end of this assignment comes from the Module PowerPoint lecture notes.



6. How was Alix, the wife of Nicholas II, related to Queen Victoria of England?

Alix is the granddaughter of Queen Victoria.⁴

7. On what chromosome is the gene that, when mutated, causes hemophilia and how does this contribute to its inheritance pattern?

X-linked recessive disease. This impacts the inheritance pattern by males only needing one X chromosome to get the disease (all boys are infected if the mother is homozygous) and girls needing both

X chromosomes to contain the allele to be infected. If the father is a carrier, all girls are carriers. If the mother is a heterozygous carrier, boys have a 50/50 chance of inheriting the disease.⁵

8. What does it mean to be a carrier for a disease?

Someone who is a carrier for a disease is someone that has one mutated allele and one normal allele. The mutated allele can be passed down to offspring in a 50/50 chance.⁶

9. Why aren't males considered carriers for hemophilia?

Males can not be considered carriers of hemophilia because they contain only one gene. There isn't another X chromosome. Males only have one X chromosome. If the female is unaffected, all of his daughters will be carriers and their sons will be unaffected. If the mother is a carrier all of his daughters have a 50/50 chance of being a carrier or affected by hemophilia (his sons will only have a 50/50 chance because of the mother).

10. In a couple of sentences, describe the physiology of the disease hemophilia. (Yes, I know it is severe bleeding because the blood cannot clot. But WHY can't the blood clot? Be very specific.)

Haemophilia can be divided into two categories: hemophilia A and B - characterized by a deficiency or absence of clotting factor VIII and IX respectively.⁷

11. What type of hemophilia (A or B) is (probably) represented in the pedigree chart?

The pedigree chart shows hemophilia B.⁸

12. Describe the mutation (at the molecular level) that apparently caused hemophilia in Alix, (and probably all of the European families that had hemophilia). Be very specific.

Czar Alexis had a causal substitution in the splice acceptor site of exon 4 in the F9 gene, which causes hemophilia B.⁸

13. How could the mutation you described in #12 result in a faulty gene product? Be very specific in your description.

The faulty gene product is caused by a causal substitution in the splice acceptor site of exon 4 in the F9 gene.⁸

14. The Romanov's son, Alexis, had hemophilia. Describe how Alexis genetically acquired hemophilia. (Use a Punnett square. You can either draw a table or line up the genotypes.)

	X ⁺	Y
X ⁺	X ⁺ X ⁺	X ⁺ Y

X^{-}	$X^{-}X^{+}$	$X^{-}Y$
---------	--------------	----------

Alexis had a 50/50 chance of being a boy or a girl because of his father's Y chromosome and a 50/50 chance of inheriting the hemophilia allele because his mother was a carrier. Ultimately, Alexis had hemophilia because he obtained the one X chromosome from his mother that had the recessive hemophilia trait.

15. Using a Punnett square (again, draw a table or line up the genotypes), explain why only males in the pedigree chart have hemophilia. (Choose at least one of the males represented in the pedigree chart, and show his parents in the Punnett square).

	X^{+}	Y
X^{+}	$X^{+}X^{+}$	$X^{+}Y$
X^{-}	$X^{-}X^{+}$	$X^{-}Y$

Only males have hemophilia because the females have a 50/50 chance of being carriers. Their mothers themselves are carriers, so the daughter has a 50/50 chance of receiving the X chromosome with the mutated allele.

16. Is it possible for a female to inherit hemophilia, and, if so, how?

It is possible for a female to inherit hemophilia. For this to occur, the mother could either be homozygous for the trait or heterozygous with the father containing the trait. If the father does not contain the trait, then his daughters can not have hemophilia. If the mother is homozygous, the daughters have a 100 percent chance of obtaining hemophilia. If the mother is heterozygous then the daughters only have a 50/50 chance of being a carrier or inheriting the disease.

17. Some historians speculate that Alexis' hemophilia condition could have led to the Russian Revolution. Explain. *You should look up the faith healer Rasputin and read about his relationship with the Romanov family.*

Molecular Analysis of People in a Mass Grave

18. Two "graves" were discovered near Yekaterinburg, Russia. Describe the number of bodies in each grave.

Nine members of the group were buried in one mass grave while two of the children were buried in a separate grave.¹

19. When were these graves discovered?

The larger mass grave with nine people was discovered in 1991. While the smaller grave with two children was discovered in the summer of 2007.¹

20. What type of testing was done to confirm the sex and familial relationships among the remains found in the mass grave?

Forensic DNA testing included: mitochondrial DNA (mtDNA), autosomal STR, and Y- STR testing.¹

21. Genetically, what does STR “stand” for? Be very specific in your answer.

STR stands for short tandem repeats that are repetitive units of 1–6 base pairs and can form up to 100 nucleotides. STRs are grouped into mono-, di-, tri-, tetra-, penta-, and hexanucleotide repeats. As the size of the repeat unit increases, the number of nucleotides in each group decreases.⁹

22. HRH Prince Philip, the Duke of Edinburgh, provided mitochondrial DNA used to identify Alix and her three daughters. HRH Prince Philip, the Duke of Edinburgh, is married to Queen Elizabeth II of England. Wait, isn’t Queen Elizabeth II related to Queen Victoria? So why was **Prince Philip’s mitochondrial DNA used**? (To help you answer this question, look at the second pedigree chart.)

Prince Philip’s mitochondrial DNA was used because mitochondrial DNA comes from the mother. So, by testing his mitochondrial DNA, the researchers were able to determine ancestry because he has a direct link to the Romanov family.



23. Who was missing from the mass grave (the one with the most skeletons)?

Tsarevich Alexei and one of his sisters.¹

Molecular Analysis of People in a Mass Grave, cont.

24. The Duke of Fife and Princess Xenia provided mitochondrial DNA used to identify Nicholas. One of these is a female and another is a male. Does that matter? What general statement can you make about their genetic relationship to Nicholas and Alexandra? Are these people still living?



25. What was discovered in the mitochondrial DNA of Nicholas that was not identified in either the Duke of Fife or Princess Xenia?

“The Duke of Fife and Princess Xenia Cheremeteff Siri, maternal relatives of Nicholas were used to reassociate the putative remains of the Tsar. A single point heteroplasmy at position 16169 (C/T=“Y”) was observed in the mtDNA sequence of the Tsar, whereas his maternal relatives were fixed for 16169 T. To confirm the authenticity of the heteroplasmy, DNA testing was conducted at the Armed Forces DNA Identification Laboratory (AFDIL) compared the mtDNA haplotype from the remains of Grand Duke

Georgij Romanov (d. 1899), brother of Tsar Nicholas II [4]. Both Tsar Nicholas II and Grand Duke Georgij Romanov shared the same point heteroplasmy at 16169 – but in differing ratios. The Tsar was mostly C/t while his brother was mostly T/c.”¹

26. What is the term given to the existence of two (or more) genetically different mitochondria in the cell?

Heteroplasmy

27. What three types of DNA were used to test the remains found in a second grave?

The three types of DNA used were mitochondrial DNA (mtDNA), autosomal STR, and Y-STR.¹

28. Of the three types of DNA you listed in #28, which one would have been used specifically to identify Alexis?

“DNA testing for 17 Y-STR markers was conducted on the remains of Tsar Nicholas II and his son, the Tsarevich Alexei. A distantly related cousin, Prince Andrew Andreevich Romanov of San Francisco, California, was used as a living relative to compare to the skeletal material.”¹

29. What was the source of the DNA used to identify Alexis?

17 Y-STR markers found in bone fragments



30. Was Anastasia in the grave in which Alexis was found?

This is still debated today, as forensic researchers are unable to determine based on DNA testing who was in the grave with Alexis.

Who Wants to Be Anastasia?

Apparently, about 200 people have wanted to be Anastasia and have claimed to be her! One of the most famous imposters was a woman named Anna Anderson (Manahan).

31. Give a brief history (2-3 sentences) of Anna Anderson-both her claims and what is thought to be true.

Anna Anderson, a Polish peasant who convinced many that she was Anastasia. With the discovery of the first grave and subsequent DNA testing, Anna Anderson was exposed as an imposter.

In conclusion, two different samples said to have come from Anna Anderson have been independently collected and analyzed by three different laboratories. This finding supports the hypothesis that Anna Anderson and Franzisca Schanzkowska were the same people.”¹¹

32. Where in the US did Anna Anderson eventually settle and why?

In 1968, Anne Anderson moved to the United States and married.¹⁰

33. What were the sources of Anna Andersons’s nuclear DNA?

“In conclusion, two different samples (hair and intestine) said to have come from Anna Anderson have been independently collected and analyzed by three different laboratories” (pdf)

34. What were the sources of Nicholas’ and Alix’s nuclear DNA?

“DNA profiles analyzed from bones of the Tsar and Tsarina” (pdf)



35. What type of analysis was done on DNA from Anna Anderson, Nicholas, and Alix?

Autosomal STR, sex-typing, YStr mtDNA analysis 10

36. Anna Anderson’s mitochondrial DNA was compared to the mitochondrial DNA of what two “other” people?

The Duke of Edinburgh and Carl Maucher



37. A hypervariable region of the mitochondrial DNA was analyzed. Define a hypervariable region.



12

38. What were the conclusions from the mitochondrial DNA comparisons?

Duke of Edinburgh disproved the maternal relationship theory. Carl Maucher may be a maternal relative of Anna Anderson

“This finding supports the hypothesis that Anna Anderson and Franzisca Schanzkowska were the same person.” 10

39. The article which describes the analysis of Anna Anderson’s DNA was published in 1995.

When were all of Nicholas’ and Alix’s children finally accounted for?

Yes, there is currently still debate on which sister was actually found in the second grave. The second grave found in 2007 contained the two missing children.



40. What did you learn from doing this assignment?

I learned a lot of cool things from this assignment. I learned about a very cool history about the Romanov family and how genetics is very important in our lives today. I learned that through researching DNA, we are able to crack the code on very interesting and important things. This is one of the reasons as to why I want to get into a genetic career.