### **Internet Resources**

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

<u>http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0004838</u> (You should be able to access the entire article. You may need to copy and paste the site address.)

<u>http://www.ncbi.nlm.nih.gov/pubmed/20557352</u> (You won't be able to access the entire article, but the abstract will give you important information.)

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.)

### <u>History</u>

1. Nicholas II was the last czar to hold power in Russia. How long had the Romanov family been in power in Russia?

For over 300 years.

- 2. Nicholas II abdicated the throne. Who took power then? Grand Duke Michael declined to accept the throne, and instead the Ural Soviets technically held reign over Russia while fighting the White Russian Army.
- 3. Describe what happened to Nicholas II and his family after he abdicated the throne? They were held in exile in Yekaterinburg, Russia. On July 17, 1918 the royal family and their staff were led to the cellar of the Ipatiev House where they were being held and executed.
- 4. 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 Russian Army was the counter-revolutionary force during the Russian Civil War.

### **Hemophilia**

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

5. How was Alix, the wife of Nicholas II, related to Queen Victoria of England? (Look at the pedigree chart very carefully.)

She is Queen Victoria's Granddaughter

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

On the X chromosome. It can only be carried by females and effects only males.

- Both Queen Victoria and Alix are designated as being carriers for hemophilia.
  - 7. What does it mean to be a carrier for a disease?
    - Although they have the mutated gene, it does not present any F8 or F9 gene deficiencies.
  - 8. Why aren't males considered *carriers* for hemophilia?

Since males do not have a second X chromosome to negate the effects of the F8 or F9 deficiencies, they can only express the mutated gene

- 9. 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.) Hemophilia is when there is a mutation in one of the genes, causing a Factor 8 or Factor 9 deficiency. This mutation changes the instructions that proteins use to make the clotting factor, thereby forming blood clots. The mutation can prevent the clotting protein from working properly or to be missing altogether. (CDC, n.d.)
- 10. What type of hemophilia (A or B) is (probably) represented in the pedigree chart? Hemophilia B
- 11. 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. There was an A>G intronic mutation 3 bp upstream of exon 4. The mutation was of the gene coding for the

coagulation at the heterzygotous stage due to a mixture of normal and mutated sequences found among female carriers.

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

This mutation is responsible for a splice anomaly, where splicing was impossible (leaving the intron mutation in the RNA and following proteins) since the mutation was so close to the exon. (Introns aren't removed, introns are translated, protein is made with faulty introns).

13. 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.)

	$\mathbf{X}^{H}$	Y	
X <sup>H</sup>	$X^H X^H$	X <sup>H</sup> Y	
	$X^H X^h$	X <sup>h</sup> Y	
X <sup>11</sup> Y is the unaffected			

father, and  $X^H X^h$  is the affected mother

14. 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.)

	$X^{H}$	Y
X <sup>H</sup>	$X^{H} X^{H}$	X <sup>H</sup> Y
X <sup>n</sup>	$X^H X^h$	X <sup>h</sup> Y

The affected individual is the bottom right square, Rupert, with Rupert's parents being a carrier mother and unaffected father. The top two squares represent Rupert's siblings, neither affected nor a carrier.

- 15. Is it possible for a female to inherit hemophilia, and, if so, how?
- Yes, if the mother is a carrier and the father is affected, the daughter could inherit the effects of hemophilia. **16.** 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 to the Romanov family.

Rasputin, as a faith healer for the imperial family, was extremely close with them, and was able to influence affairs of the state indirectly. As Rasputin grew more unpopular, so did the imperial family by association, leading to the Russian Revolution, and the fall of the Romanov dynasty.

### Molecular Analysis of People in a Mass Grave

17. Two "graves" were discovered near Yekaterinburg, Russia. When were these graves discovered, and how many bodies were found in each grave?

The first grave was discovered in the late 1970s with 5 of the seven members of the royal family and their four servants. The second grave was found in the summer of 2007 approximately 70 meters from the first grave, this contained two more sets of bones.

- 18. What type of testing was done to confirm sex and <u>familial relationships</u> among the remains found in the mass grave? If you use an abbreviation, write it out and define what it is. The sex was determined by the sciatic notch dimensions. The familial relationships were determined by Mitochondrial DNA testing was completed on both the bones in the second grave, and 4 sets of remains from the first grave, which when combined with DNA quantification gave familial relationships.
- 19. 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 is the Great-Great-Great Grandson of Queen Victoria, and is more closely related to the Romanovs than Queen Elizabeth II of England.
- 20. Who was missing from the mass grave (the one with the most skeletons)? Alexei and one of his sisters (Anastasia/Maria).

### Molecular Analysis of People in a Mass Grave, cont.

21. 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?

They are maternal relatives (Related by mother's lineage). The gender does not matter for Mitochondrial DNA.

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

A single point heteroplasmy at position 16169 was observed in the mtDNA sequence.

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

- 24. What three types of DNA were used to test the remains found in a second grave? Again, if you use an abbreviation, write it out and define what it is. Mitochondrial DNA, Nuclear DNA, and Autosomal DNA.
- 25. Of the three types of DNA you listed in the previous answer, which one would have been used specifically to identify Alexis and why?
  Autosomal DNA, since it was the Y-STR testing of the teeth that determined the match of the second grave's remains to Tsar Nicholas II.
- 26. Was Anastasia in the grave in which Alexis was found?

It cannot be determined whether it is Anastasia or Maria in the grave from the results that were given.

### 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).

- 27. Give a brief history (2-3 sentences) of Anna Anderson-both her claims and what is thought to be true. Anna Anderson showed up around 1920 and claimed to be Duchess Anastasia, daughter of Tsar Nicholas II. The believers say that she escaped execution from the Bolsheviks. Disbelievers say that she is Franzisca Schanzkowska, a German who was badly injured in a munitions factory in the first world war and disappeared from a mental hospital in 1920, around the same time "Anastasia" appeared.
- 28. Where in the US did Anna Anderson eventually settle and why? She settled in Charlottesville, VA when she married Professor J.E. Manahan.
- 29. What were the sources of Anna Andersons's nuclear DNA? Bowel samples from her surgery
- 30. What were the sources of Nicholas' and Alix's nuclear DNA? The bones of the Tsar and Tsarina.
- 31. What type of analysis was done on DNA from Anna Anderson, Nicholas, and Alix? Short tandem repeat (STR) analysis.
- 32. Anna Anderson's mitochondrial DNA was compared to the mitochondrial DNA of what two "other" people? The Duke of Edinburgh and Carl Maucher.
- 33. A hypervariable region of the mitochondrial DNA was analyzed. Define a hypervariable region. A hypervariable region is a portion of mtDNA that has a repeating set of Nucleotides.
- 34. What were the conclusions from the mitochondrial DNA comparisons? That Carl Maucher and Anna Anderson are related, and that Anna Anderson and Franzisca Schanzkowska are the same person.
- 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? 1991 (Katz, B., 2018)
- 36. What was the most surprising thing that you learned from doing this assignment? Queen Victoria is related to multiple royal families.

Katz, B. (2018, July 17) DNA Analysis Confirms Authenticity of Romanovs' Remains. Smithsonian Magazine. https://www.smithsonianmag.com/smart-news/dna-analysis-confirms-authenticity-remains-attributed-romanovs-180969674/

Are you still interested in the life of the last Tsar of Russia and his relationship to British royalty? The headline for the following article showed up on my Internet browser earlier this year. While I can't vouch for it as it did not appear in a peer-reviewed journal, it might be interesting reading for you.

https://www.townandcountrymag.com/society/tradition/a31028924/windsors-romanovs-relationship-last-gathering-true-story/



Source for the pedigree chart above: Janet Stein Carter, Biology Instructor at Clermont College, University of Cincinnati