Internet Resources

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

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.)*

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.)*

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.)

History

1. Nicholas II was the last Romanov to hold power in Russia. What was his title?
   Nicholas II the last ruling Russian Tsar.
2. How long had the Romanov family been in power in Russia?
   For over 300 years.
3. Nicholas II abdicated the throne. Who took power then?
   Nicholas II wanted his brother to assume power however his brother, Grand Duke Michael, declined. The Russian Provisional Government took control over Russia after Nicholas II.

https://courses.lumenlearning.com/suny-hccc-worldhistory2/chapter/the-provisional-government/

4. What happened to Nicholas II and his family after he abdicated the throne?
   They were executed by the Ural Soviets for the belief that the will of the people loyal to the Tsar would be broken with the death of Nicholas II and is family.
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. Their political and military effectiveness was hamstrung by divided leadership, disparate motives and inability to offer hope for the future.

https://alphahistory.com/russianrevolution/white-armies/

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? (Look at the pedigree chart carefully.)
   Alix is the granddaughter of Queen Victoria of England. Queen Victoria was married to Albert.

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

Both Queen Victoria and Alix are designated as being carriers for hemophilia.

The chromosome that causes hemophilia is the X chromosome. Hemophilia is an X-linked recessive disease; thus, its inheritance pattern typically skips females and will be seen in about every other male.

8. What does it mean to be a carrier for a disease?
   To be a carrier for hemophilia is to be a **female** that has **one wild type** (heathy, normal) allele on one of their X chromosomes and to have **one mutant** (disease causing) allele on their other X chromosome.
9. Why aren't males considered carriers for hemophilia?

For males only have one x chromosome. Males can either have the disease (a mutant allele on their one X chromosome) or they can either have a normal, wildtype allele on their sole X chromosome—there is no in-between.

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.)

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

B

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.

The mutation at the molecular level is caused by a causal substitution in the splice acceptor site of exon 4 in the F9 gene. This mutation that is responsible for hemophilia B had traumatized European royal families throughout the 20th century.

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

It would result in a loss of function. Because the protein is not being made thus a difficency in clotting factor IX is a result.

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 genetically acquired hemophilia for his mother was a carrier. Due to his mother being a carrier, and the nature of inheritance with the disease of hemophilia, all male children of his mother (regardless of the father) would have a 50% chance of having the disease and a 50% chance of not inheriting the disease.

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

In this example (Alexis's parents), all daughter females would not have the disease. 50% of the daughter females will have two wild type normal alleles, and the other 50% of all of the daughters would simply just be carriers for the disease. In this pedigree, it is only possible for males to have hemophilia for there are no females with the disease. Males only need one mutant allele to have the disease for males only have one X chromosome, but females need two mutant alleles to have the X-linked recessive gene for they have two X chromosomes. All females in this pedigree would thus only ever be at worst a carrier.

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

Yes, it is possible for a female to inherit hemophilia. The conditions that are utterly necessary for a female to inherit the genetic disease of hemophilia are that the mom is either a carrier or has the disease and that the dad must have 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 to the Romanov family.***

Alexis' hemophilia condition is speculated to have led to the Russian Revolution. His disease is thought to have done this because due to his disease his family was introduced to the faith healer Rasputin who promised the Romanov family that he could heal Alexis' hemophilia. Then various politicians and journalists used Rasputin's association with the imperial family to undermine the dynasty's credibility and push for reform. Thus, giving that necessary spark for the Russian Revolution to kickstart.

Molecular Analysis of People in a Mass Grave

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

The first grave was described as a mass grave for there was a total of nine people in this grave. This mass grave had five members of the Romanov family and four servants of the family. The second grave that was discovered only had two people—Alexis and one of his sisters (either Anastasia or Maria).

19. When were these graves discovered?

The first mass grave was discovered in the late 1970's but was kept as a secret until 1991. In 1991 the grave was "officially discovered." The second smaller two-person grave was discovered in the summer of 2007.

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

Nuclear DNA testing of Five STR markers confirmed the sex of the skeletons and established familial relationships among the remains of the Tsar, the Tsarina, and three of the daughters recovered from the grave.

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

Short Tandem Repeats (involve a repetitive unit of 1-6 base pairs) (used in forensic science for their polymorphisms and high mutation rates).

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 he is also a direct maternal relative of the Romanov family.

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

Two children of Nicholas II— Alexis and one of his sisters (either Anastasia or Maria).

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?

It does not matter that one was female, and one was male. Children always get their mitochondria from their mother. Both the Duke of Fife and Xenia would and should have the same mitochondrial DNA since they are distant maternal relatives. These two people should have the same mitochondria as Nicholas since all preceding generations of all three people are all directly related to the females of this family through females (each member was born from a mother that was born from a mother that was born from a mother within this family etc.). Xenia and Fife are not genetically related to Alexandra and all four of these people are dead.

25. 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 (C/T="Y") was observed in the mtDNA sequence of the Tsar, whereas his maternal relatives were fixed for 16169 T.

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?
mtDNA (mitochondrial DNA), autosomal STR (autosomal DNA), and Y-STR (sex chromosomal DNA).

28. Of the three types of DNA you listed in #28, which one would have been used specifically to identify Alexis?
Y-STR (sex chromosomal DNA)

29. What was the source of the DNA used to identify Alexis?
17 Y-STR markers found in bone fragments.

Table 1

Sequences of the samples recovered from "Grave #2" in August 2007 and tested in this study.

Bone	Russian #	Region Sequenced	Sequence
Right humerus	141	16024-16391 and 35-369	16111T, 16357C, 263G, 315.1C
Occipital fragment	139	no results	–
Occipital fragment	144.1	16024-576	16111T, 16357C, 16519C, 263G, 315.1C, 524.1A; 524.2C
Right os coxae-♀	145	16024-16391 and 35-369	16111T, 16357C, 263G, 315.1C
Left femur	146.1*	16024-576	16111T, 16357C, 16519C, 263G, 315.1C, 524.1A; 524.2C
Right femur - ♀	147*	16024-576	16111T, 16357C, 16519C, 263G, 315.1C, 524.1A; 524.2C
Right scapula	140	16024-16391 and 35-369	16111T, 16357C, 263G, 315.1C
Cranial fragment	143	16024-16391 and 35-369	16111T, 16357C, 263G, 315.1C
Left ilium	142	no results	–

Samples marked with an asterisk (*) were tested by AFDIL and GMI.
doi:10.1371/journal.pone.0004838.t001

30. Was Anastasia in the grave in which Alexis was found?
That is still unknown. It is still debated to be either be her or Maria.

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 is a polish peasant who made many believe that she was Anastasia (a potential missing daughter of Nicholas II). Anna was committed to an asylum at one point in her life. She was later proved an imposter through DNA testing.

32. Where in the US did Anna Anderson eventually settle and why?
Anna Anderson eventually settled in Charlottesville, Virginia after she married a history professor.

33. What were the sources of Anna Andersons's nuclear DNA?
Hair and intestine.

34. What were the sources of Nicholas' and Alix's nuclear DNA?
Their skeletons (bones—for instance a tooth of Nicholas).

35. What type of analysis was done on DNA from Anna Anderson, Nicholas, and Alix?
Autosomal STR analysis, sex typing using the amelogenin test, Y-STR analysis, and mtDNA analysis.

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.
A hypervariable region are two non-coding regions of the mitochondrial genome each with approximately 300 base pairs that flank the origin of replication. These two regions show the most variation (SNPs and InDels) among different [people. The DNA in these regions accumulate mutations are ten times the rate of DNA sequences in the nuclear genome.

38. What were the conclusions from the mitochondrial DNA comparisons?
Duke of Edinburg —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.

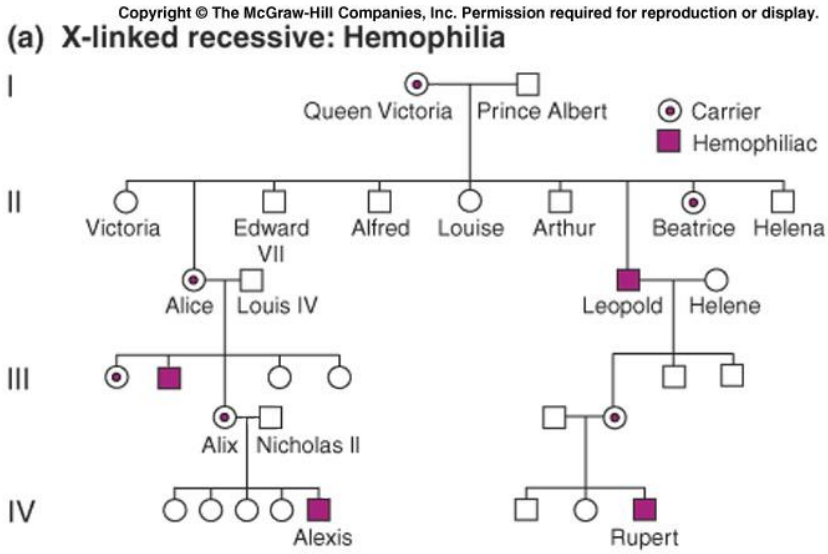
39. The article which describes the analysis of Anna Anderson's DNA was published in 1995.
When were all of Nicholas' and Alix's children finally accounted for?

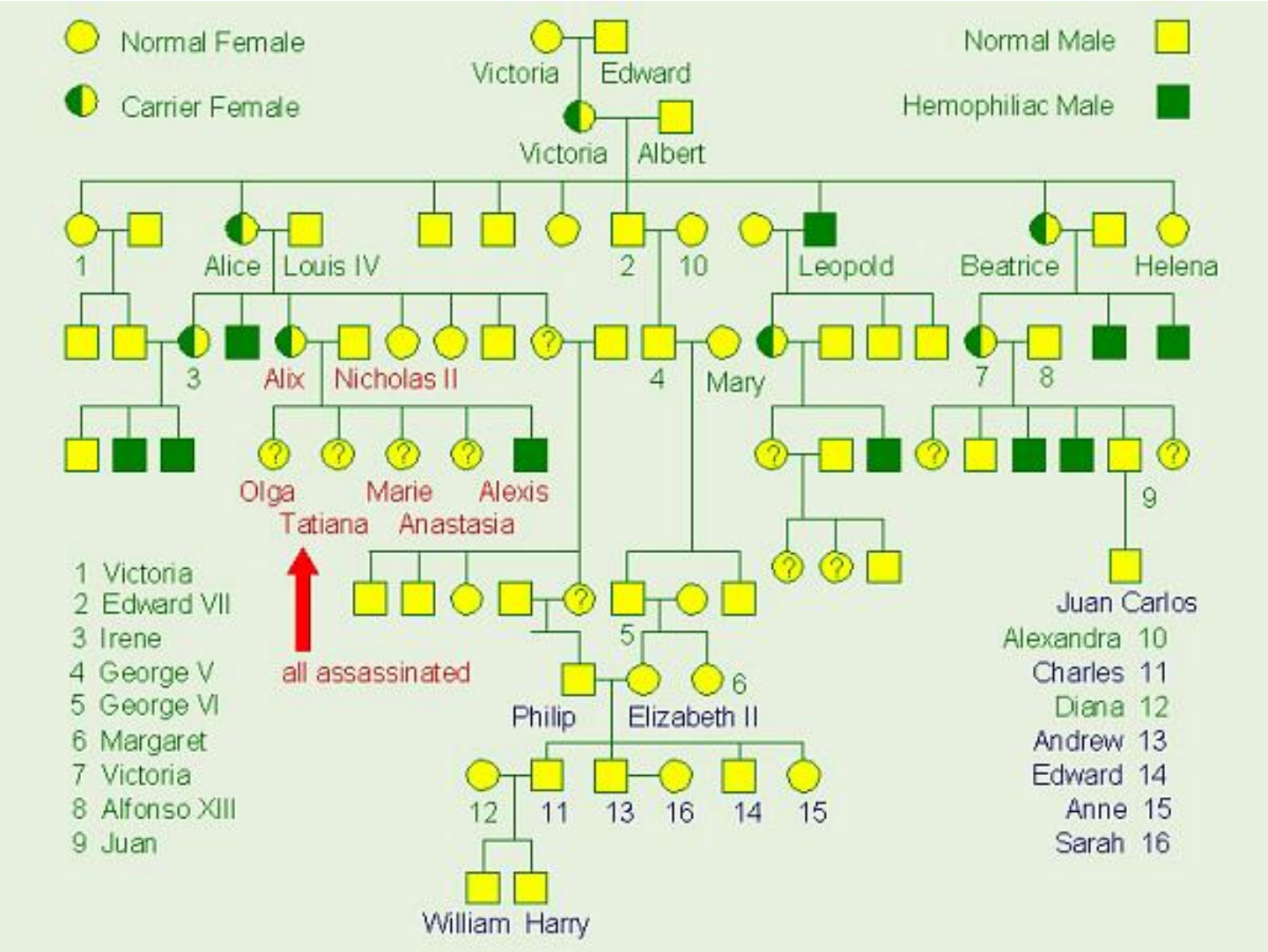
Yes, all of the children are "finally" accounted for. There is irrefutable evidence that the two individuals recovered from the 2007 grave are the two missing children of the Romanov family: the Tsarevich Alexei and one of his sisters.

40. What did you learn from doing this assignment?

I learned a lot about the Romanov family. I learned how to apply this classes material to a real-life instance. I learned that hemophilia run in the royal family too.

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