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 czar to hold power in Russia. How long had the Romanov family been in power in Russia?
   - By 1917, the Romanov family had been in power, ruling the country of Russia for over 300 years.
2. Nicholas II abdicated the throne. Who took power then?
   - Nicholas II had abdicated the throne to his brother, Grand Duke Michael, however he declined. With his declination for his brother's crown, in his place a provincial government was installed.
3. Describe what happened to Nicholas II and his family after he abdicated the throne?
   - After abdicating his throne, Nicholas II, his wife, and five children were exiled to Yekaterinburg, Russia. Additional people who were there with them were four loyal staff members of the family (physician, valet, maid, and cook). Unfortunately, it was not long until the family and their staff were executed in the basement of the Ipatiev House where they were living in exile.
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 were all those who were loyal to Czar Nicholas II. Those in opposition were the Red Russian Army, who wanted communism.

Hemophilia

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

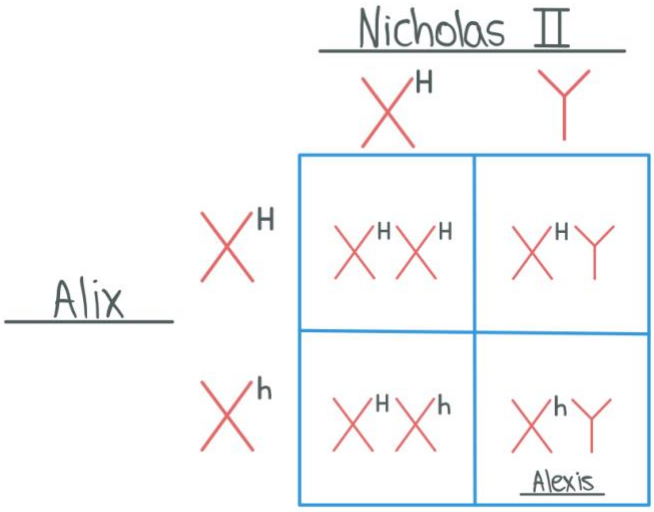
5. How was Alix, the wife of Nicholas II, related to Queen Victoria of England? **(Look at the pedigree chart very carefully.)**
   - Queen Victoria was Alix's maternal grandmother.
6. On what chromosome is the gene that, when mutated, causes hemophilia, and how does this contribute to its inheritance pattern?
   - The gene which when mutated, causes hemophilia is found on the X chromosome. This contributes to the inheritance pattern, as males mostly have hemophilia since they only receive one X chromosome (meaning one mutant allele is enough to cause the disease). Females are carriers, and do not get hemophilia as often as males since they receive two X chromosomes (therefore needing both chromosomes to possess mutant alleles in order to have the disease).

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

7. What does it mean to be a carrier for a disease?
   - To be a carrier for a disease means that the person themself does not have the disease but carries the mutant allele for the disease which can be passed on to the child. Women are carriers for hemophilia when they have one X chromosome with the normal allele, and the one X chromosome with the mutant allele (normal allele refers to clotting factor, while mutant allele refers to mutated clotting factor gene).
8. Why aren't males considered *carriers* for hemophilia?
   - Males are not considered carriers for hemophilia, as this is a disease caused by mutation in a X chromosome gene. Since males only possess one X chromosome, they either have the disease (if they happen to receive the mutated allele from their mother) or they don't (meaning they received the normal allele from their mother). Carriers have <u>both</u> a mutated allele on one chromosome and a normal allele on the other; while males have one or the other but <u>not both</u>.
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.)**

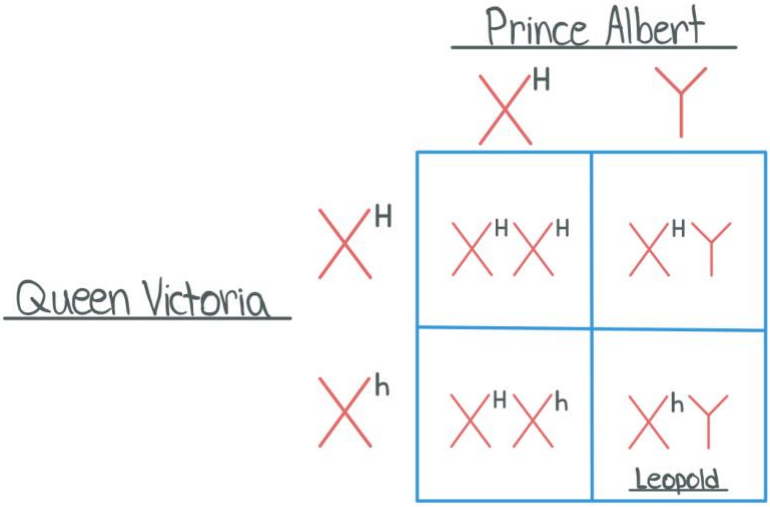
- When there is a mutation in the gene found on the X chromosome which gives instructions for making clotting factor proteins (which necessary to form blood clots) it results in hemophilia. The mutation or change in the gene can prevent the clotting factor proteins from working correctly, or they could be completely absent. (There is clotting factor VIII and IX)

10. What type of hemophilia (A or B) is (probably) represented in the pedigree chart?
    - Hemophilia B is probably represented in the pedigree chart, despite Hemophilia A being about four times as common as 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.
    - The mutation to cause the disease was revealed as being a substitution in the splice acceptor site of exon 4 in the F9 gene.
12. How could the mutation you described in #12 result in a faulty gene product? Be *very* specific in your description.
    - Substitution in the splice acceptor site means that a different nucleotide is put in place. A different nucleotide put in place therefore creates a different codon, coding for a different amino acid, and subsequently a completely different protein to be produced. Whereas if no mutation occurred a clotting factor protein would be coded for and produced, meaning no hemophilia.
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.)
    - Alexis genetically acquired hemophilia as he received the Y chromosome from his father, and the X chromosome he received from his mother was the one which had the mutated gene, therefore receiving the hemophilia allele.



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.)
    - Only males in the pedigree chart have hemophilia due to only having on X chromosome. For a female to have hemophilia, her mother must be a carrier AND her father must have hemophilia. As it can be seen in the pedigree chart, none of the parents consisted of both a female carrier and a male with hemophilia, it is

only one or the other; therefore, only males in the pedigree chart have hemophilia.



15. Is it possible for a female to inherit hemophilia, and, if so, how?
    - Yes, it is possible for a female to inherit hemophilia. For this to occur, she must receive the X chromosome with mutant clotting factor gene from her mother (who is a usually a carrier, but could have hemophilia herself), as well as the X chromosome with mutant clotting factor gene from her father (meaning he has the disease himself).
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.***
    - With traditional doctors unable to help Alexis's suffering from hemophilia, his mother Alix turned to Rasputin, an unorthodox healer despised and feared by many of the country's elites. Alix was a believer, as she truly thought he was providing her son relief. Rasputin's bond with the family grew as he continued to successfully treat the prince. The Romanov family already struggled for support, but as Rasputin became more involved in the family, their popularity decreased. All of this got much worse as time went along. It is because of the prince's hemophilia condition that Rasputin became involved with the family (contributing to their downfall), which is why some historians say his condition led to the Russian Revolution.

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 mass grave was located in the late 1970s, it was discovered to contain the remains of five out seven family members and staff there were. Despite efforts, it was not until the summer of 2007 that the second mass grave was located, in it were the remains of two people.
18. What type of testing was done to confirm sex and familial relationships among the remains found in the mass grave? If you use an abbreviation, write it out and define what it is.
    - Three different types of tests were done to confirm sex and familial relationships abount the remains found in the mass graves: Mitochondrial DNA Testing (mtDNA), Autosomal STR Testing, and Y-STR Testing (STR = Short Random Repeat). Mitochondrial DNA testing is used to uncover people's mtDNA haplotype from which people's maternal line descends. This test can be run both males and females as mothers pass mitochondria to all their children. Autosomal STR testing is used to determine someone's autosomal chromosomes, containing DNA with everyone they are related to, both maternal and paternal. Y-STR testing is used to uncover male's Y-chromosome haplogroup from which their paternal line descends. All of the tests were used to help get a full picture of relations and sex of the remains.
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 Phillip's DNA was used, as he possessed the same mitochondrial maternal lineage back to Queen Victoria, as Alix and her three daughters did. In other words, it is the maternal side of Prince Phillips from which he descends from Queen Victoria, same for Alix and her daughters. In contrast, Queen Elizabeth descends from Queen Victoria from her paternal side.

20. Who was missing from the mass grave (the one with the most skeletons)?
    - Following DNA testing and analysis it was discovered that the two individual's remains who were missing from the first discovered mass grave—but found in the second grave in 2007—was two of the Romanov family children, their son Alexis, and one of his sisters. It is debated which sister it was though, with Russian experts saying Maria, while US experts saying Anastasia.

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?
    - It did not matter that the Duke of Fife was a male and Princess Xenia was a female. What mattered, was that they both were maternal relatives of Nicholas, so mtDNA testing could be used.
22. What was discovered in the mitochondrial DNA of Nicholas that was not identified in either the Duke of Fife or Princess Xenia?
    - The mitochondrial DNA sequence of Nicholas, the Duke of Fife, and Princess Xenia matched, except for a difference in a single point heteroplasmy. For the Tsar, the single point heteroplasmy at position 16169 was (C/T = "Y"); while his maternal relatives had T at this position.
23. What is the term given to the existence of two (or more) genetically different mitochondria in the cell?
    - The term for heteroplasmy describes the existence of two (or more) genetically different mitochondria in the cell.
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.
    - Three different types of tests were done for both the first and second graves to confirm sex and familial relationships about the remains. These tests were: Mitochondrial DNA Testing (mtDNA), Autosomal STR Testing, and Y-STR Testing (STR = Short Tandem Repeat). Mitochondrial DNA testing is used to uncover people's mtDNA haplotype from which people's maternal line descends. This test can be run both males and females as mothers pass mitochondria to all their children. Autosomal STR testing is used to determine someone's autosomal chromosomes, containing DNA with everyone they are related to, both maternal and paternal. Y-STR testing is used to uncover male's Y-chromosome haplogroup from which their paternal line descends.
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?
    - The type of DNA used to identify Alexis was that of the Y-chromosome. Being the son of Nicholas, he would have received his Y-chromosome directly from him. With this being said, Y-STR testing could be completed to identify if the haplotypes matched in each male, which they did.
26. Was Anastasia in the grave in which Alexis was found?
    - It cannot be determined based off of DNA results which sister was in the grave with Alexis (Russian experts believe Maria, while US experts believe Anastasia). Due to not having specific DNA to reference from each individual sister, researchers using DNA profiling could only conclusively identify that the male remains were Alexis.

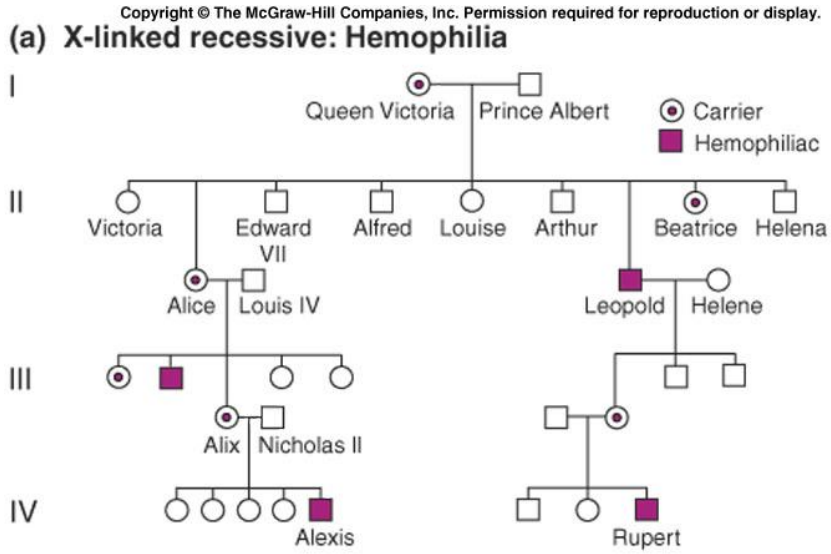
Who Wants to Be Anastasia?

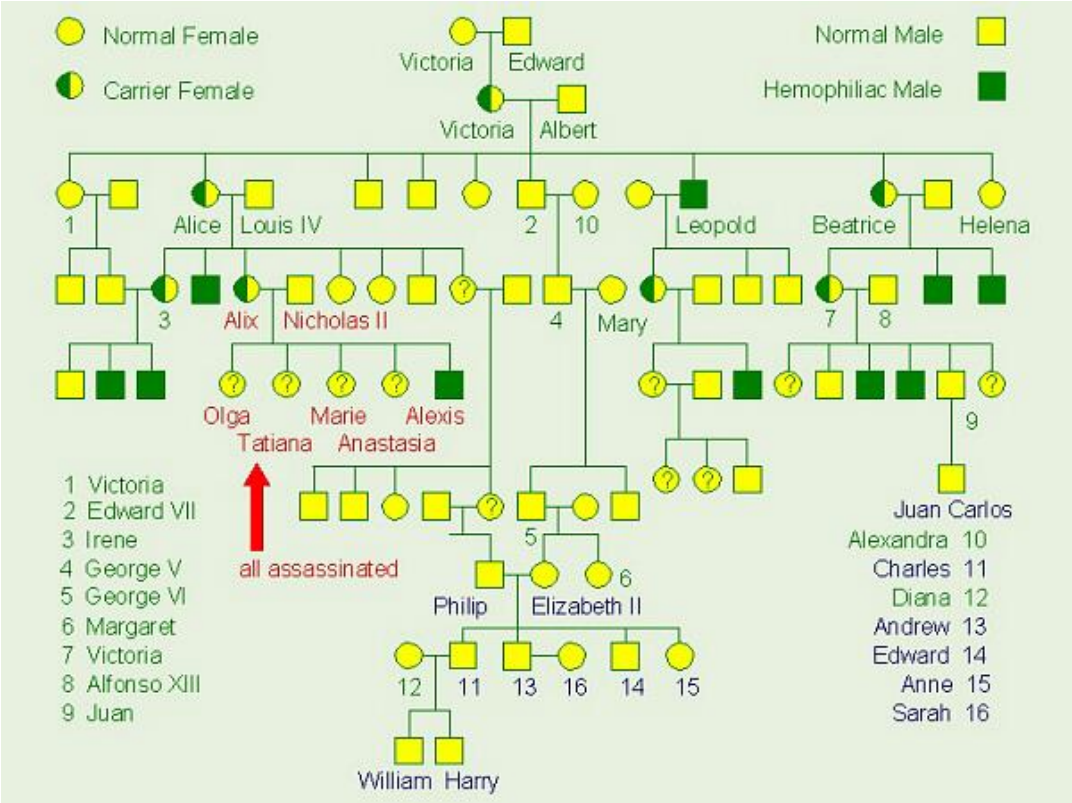
Apparently, about 200 people have wanted to be Anastasia and have claimed to be her! One of the most famous imposters was a woman named Anna Anderson (Manahan).

27. Give a brief history (2-3 sentences) of Anna Anderson-both her claims and what is thought to be true.
    - A controversy lasting almost ¾ of a century surrounded who Anna Anderson truly was. She claimed to be the Royal Duchess Anastasia, the daughter of Nicholas II and Alix. One theory from those who did not believe her said that Anna was someone named Franzisca Schanzkowska. All a detetective uncovered was this woman was injured in WWI Berlin, admitted to two mental hospitals, and then disappeared in 1920 (around the time that Anna made her claim). It was thought by many that Anderson and Schanzkowska were the same woman.
28. Where in the US did Anna Anderson eventually settle and why?
    - Charlottesville, Virginia is where Anna Anderson eventually settled after marrying an American history professor.
29. What were the sources of Anna Andersons's nuclear DNA?
    - The sources of Anderson's nuclear DNA were intestine (specifically small bowel) and hair samples.

30. What were the sources of Nicholas' and Alix's nuclear DNA?
    - The sources of Nichola's and Alix's nuclear DNA were from their bones.
31. What type of analysis was done on DNA from Anna Anderson, Nicholas, and Alix?
    - Both STR (Short Tandem Repeat) and mtDNA (Mitochondria DNA) analysis was done on DNA from Anna Anderson, Nicholas, and Alix.
32. Anna Anderson's mitochondrial DNA was compared to the mitochondrial DNA of what two "other" people?
    - Anderson's mitochondrial DNA was compared to the mitochondrial DNA of HRH Duke of Edinburgh (great nephew of Alix) and Carl Maucher (great nephew of Schanzkowska).
33. A hypervariable region of the mitochondrial DNA was analyzed. Define a hypervariable region.
    - A hypervariable region of mitochondrial DNA has high rates of mutation, leading to polymorphisms such as nucleotides being substituted. The genetic variation of these regions are used to determine disease causing genes and also therefore can be used in testing for relations of people.
34. What were the conclusions from the mitochondrial DNA comparisons?
    - When comparing Anderson's mitochondrial DNA to the mitochondrial DNA of the great nephew of Alix, six differences in the hypervariable region were discovered. This finding disproving her relation to the royal family. As for comparing Anderson's mitochondrial DNA to Carl Maucher's, a match was observed between their samples. This finding suggested that Maucher and Anderson were likely maternal relatives (supporting the idea that Anna Anderson and Franzisca Schanzkowska were the same woman).
35. 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?
    - All of Nicholas's and Alix's children were finally accounted for in 2007 when the two missing children were discovered. It was found to be their son Alexis, and one of their daughters, but specific identity of the daughter could not be concluded.
36. What was the most surprising thing that you learned from doing this assignment?
    - I enjoyed learning about the aspects of both history and genetics with this assignment. What surprised me was how Russian royalty was also part of Queen Victoria's descendants and family! I have always just thought of her descendants being British Royalty. It is interesting to think about how if things were different, and the Romanov family continued to rule Russia, how different relations between nations and conflicts that have since happened may have been.

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

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