Old Dominion University/BIOL294-Genetics/Rinehart-Kim/Romanov Project

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BIOL294
Dr. Rinehart-Kim

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.)

Outside Resources

History Editors. et al. Women claiming to be Anastasia Romanov arrives in the U.S. *History*; https://www.history.com/this-day-in-history/anastasia-arrives-in-the-united-states

Stoneking, Mark. Et al. Hypervariable Sites in the mtDNA Control Region Are Mutational Hotspots. *PubMed Central*; https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1287875/

Alpha History Editors. et al. The White Armies. *Alpha History*; https://alphahistory.com/russianrevolution/white-armies/

Britannica Editors. et al. Grigori Rasputin. *Britannia*; https://www.britannica.com/biography/Grigory-Yefimovich-Rasputin

History

1. Nicholas II was the last czar to hold power in Russia. How long had the Romanov family been in power in Russia?
   The Romanov family ruled the country of Russia for over 300 years. This was until the Bolshevik revolution in 1917 as Nicholas II and his family were executed.
2. Nicholas II abdicated the throne. Who took power then?
   Nicholas II abdicated the throne in favor of his brother George Grand Duke Michael, but he declined to accept the throne.
3. Describe what happened to Nicholas II and his family after he abdicated the throne?
   After Nicholas II addicted the throne, him, and his family (his wife and their five children) were exiled and sent to Yekaterinburg, Russia. The family and also four loyal members of their staff were taken to the cellar of the Ipatiev House, where they were all 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 was a counter-revolutionary group that was part of the Russian Civil War. They were part of the socialist movement in Russia that fought against the Red Russian Army.

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 the granddaughter to Queen Victoria of England. She is the daughter of Alice, daughter of Queen Victoria, and Louis IV.

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

The chromosome in the gene that causes hemophilia is an X-linked recessive chromosome. Since males only have one X-chromosome, when having the X-recessive gene they will have hemophilia. Women have two X-chromosomes, so when they have the recessive X-gene, they won't have hemophilia, but they can carry the X-recessive gene and pass it on to next generations. Since it can be carried, it can be used to track maternal relationships.

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 means that you have the capability to transfer the disease/mutation to generations, but you might not show any symptoms. This usually means that you are carrying a recessive gene, but also a dominant one so therefore you are not affected by the mutation.

8. Why aren't males considered *carriers* for hemophilia?

Since males only have one X-gene, when they receive a recessive X-gene, they will have the symptoms of that gene. Since hemophilia is a X-linked recessive gene, when the males have it, they will have hemophilia.

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 the blood disease that is caused by low levels of factor VIII and IX which secrete the proteins for clotting. With low levels of clotting factors, there is nothing stopping continuous bleeding which results hemophilia. All the clotting factors work with platelets to in the coagulation cascade.

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

Hemophilia B is most likely represented in the pedigree chart.

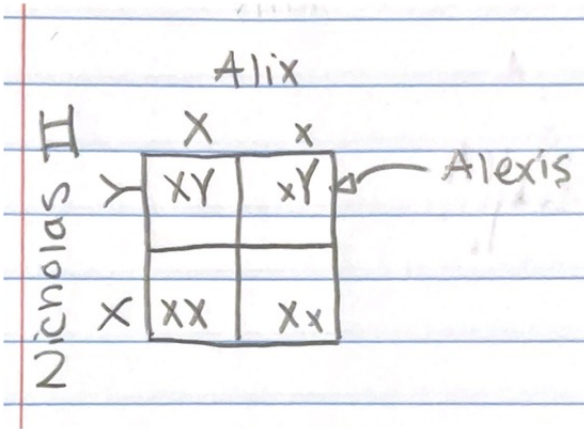
11. Describe the mutation (at the molecular level) that apparently caused hemophilia in Alix, (and probably all of the European families that had hemophilia). Be *very* specific.

The mutation that caused the hemophilia in Alix was due to a substitution at the acceptor site exon 4 in the F9 gene. The substitution happens between the nitrogen bases that change the exons causing a change in the amino acid polypeptide sequence.

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

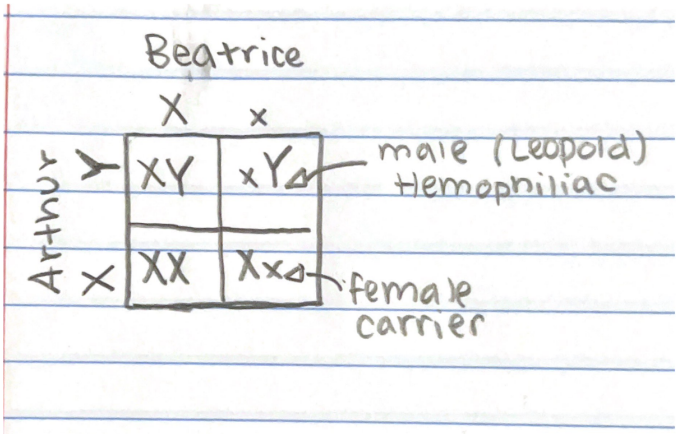
The mutation causes a splicing in the gene that is responsible for the clotting factor proteins that help the stop of bleeding. This mutation causes the low levels of factor VIII and factor IX which is what determines the severity of hemophilia. Without these proper factors, the clotting of blood will decrease which can result in fatality.

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 acquired hemophilia through is mother recessive X-gene as she was a carrier.

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 can get hemophilia in the chart since they only carry one X-gene. When they get the recessive X-gene from their mother, then they get hemophilia. A female could get it if they had two recessive X-genes, but that's not the case for this chart.

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

Yes, the female can inherit hemophilia, but it is extremely rare for them to exhibit the symptoms and actually be a hemophiliac. This is due to the need to have two recessive X-genes which is unlikely. Most females will be carriers as they will only have one recessive X-gene.

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.***

Grigori Rasputin, the faith healer, was to improve the condition of Aleksey's hemophilia as he was the heir to throne. This made him an influential favorite to Nicholas II and Empress Alexandra. Many people didn't like his influence in the government, so they decided to assassinate him and revolt against the Romanov family. He was known as a madman and since the Romanov family gave him so much influence, it discredited their power.

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 found in 1991, which was the larger grave site that held most of the family. There were nine bodies that were found in the grave. The second grave was found in 2007 by an amateur archeologist which had two of the missing children.

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.

Nuclear DNA testing of five STR markers were used to confirm the sex of the skeletons and also to establish a familial relationship with the remains of the Tsar, the Tsarina, and also their three children (daughters).

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.)

To keep it simple, they are related. Prince Phillip, the Duke of Edinburgh, and Queen Elizabeth II are distant cousins. Because of this they both are related to the Romanov family, they can be tested, but Prince Phillip is closer related to the Romanov family.

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

One of the two sisters/daughters and the son, Tsarevich Alexei. Since it's only bones and they both were young, they were able to determine the sex, but they were not able to tell which sister was missing.

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?

One being a female and the other being a male only matters when it comes to determining and identifying the parents, but since it is just comparing mtDNA, it does not matter. The mtDNA shows that there are familial relationships with the family from a female or matriarch. The Duke of Fifi and Princess Xemia mtDNA has been passed down to Nicholas II which proves that they are maternal relatives.

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

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

Heteroplasmy.

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.

Nuclear STR, mitochondrial and ancient DNA. STR is short tandem repeat analysis.

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?

Mitochondrial because they were able to use the mtDNA from the tooth remains of Tsar Nicholas II. They used his specific mtDNA and matched it with HVI and HVII and assess the frequency of the haplotype from the Tsar.

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

It was not possible to tell if it was Anastasia or Maria found with Alexis or not because they were only tell sex and age, but it was concluded that one was in the large grave and the other was 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).

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

Anna Anderson Manahan claimed that she was Duchess Anastasia, daughter of Tsar Nicholas II and that she escaped her execution. Since one of the daughter's bodies was not found at the time, this allowed many people to actually believe her claim. Many thought that since the whole family was buried together and only possibly Anastasia (did not know which sister was buried) and one her brothers were not found that she could have possibly escaped the execution.

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

In 1968, Anna Anderson got married to a history professor in America, J.E. Manahan. This caused to her to move to Charlottesville, Virginia where she settled and lived the rest of her life.

29. What were the sources of Anna Andersons's nuclear DNA?

There were bowel samples (hair and intestine) removed from Anne Anderson at the Martha Jefferson Hospital in Charlottesville, Virginia.

30. What were the sources of Nicholas' and Alix's nuclear DNA?'

The bones/teeth of the Tsar and Tsarina were used as sources for the DNA of Nicholas and Alix. They also got a blood sample from the Duke of Edinburgh who is a direct maternal descendant of Tsarina.

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

The Forensic Science Service (FSS) did a short tandem repeat (STR) analysis using a quadruplex and mtDNA analysis using a modification of a solid protocol. Also, AFIP performed a mitochondrial (mt) DNA analysis using dye-terminator cycle sequencing.

32. Anna Anderson's mitochondrial DNA was compared to the mitochondrial DNA of what two "other" people?

They received a blood sample from HRH the Duke of Edinburgh, who is a direct maternal descendant of the Tsarina. The other person who donated blood is Carl Maucher, great nephew of Franzsica Schanzkowska, a believed suspect of Anna Anderson's true identity.

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

Hypervariable sites in mtDNA are the representation of mutational hotspots. It is also said that hypervariable sites reflect ancient mtDNA mutations that have been adjusted in different lineages via recombination. The literal definition is it being a short variable polypeptide segment of an antibody.

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

When comparing Anna Anderson directly to Tsar and Tsarina; there were four out of five STRs that were inconsistent with the idea that Anna was their child. Also, when comparing Anna to the Duke of Edinburgh, there was six differences in hypervariable region 1, which again disproved maternal relation. It was concluded that the hypothesis that Anna Anderson and Franzisca Schanzkowska being the same person was supported by the testing of comparison of Anna and Carl Maucher.

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?

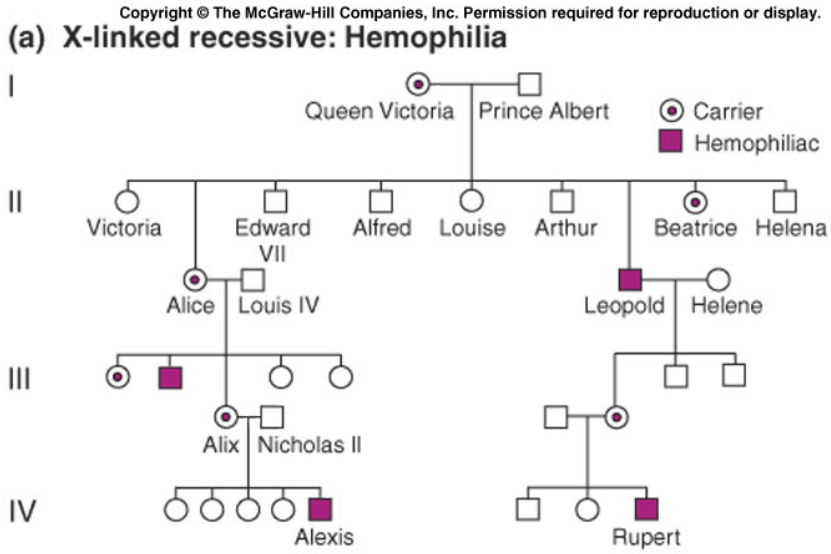
In the summer of 2007, the group of amateur archeologists found the second grave around 70 meters away from the larger grave with the Romanov family. With DNA testing of material from the 1991 grave, it was concluded that the two individuals are indeed the two missing children of the Romanov family.

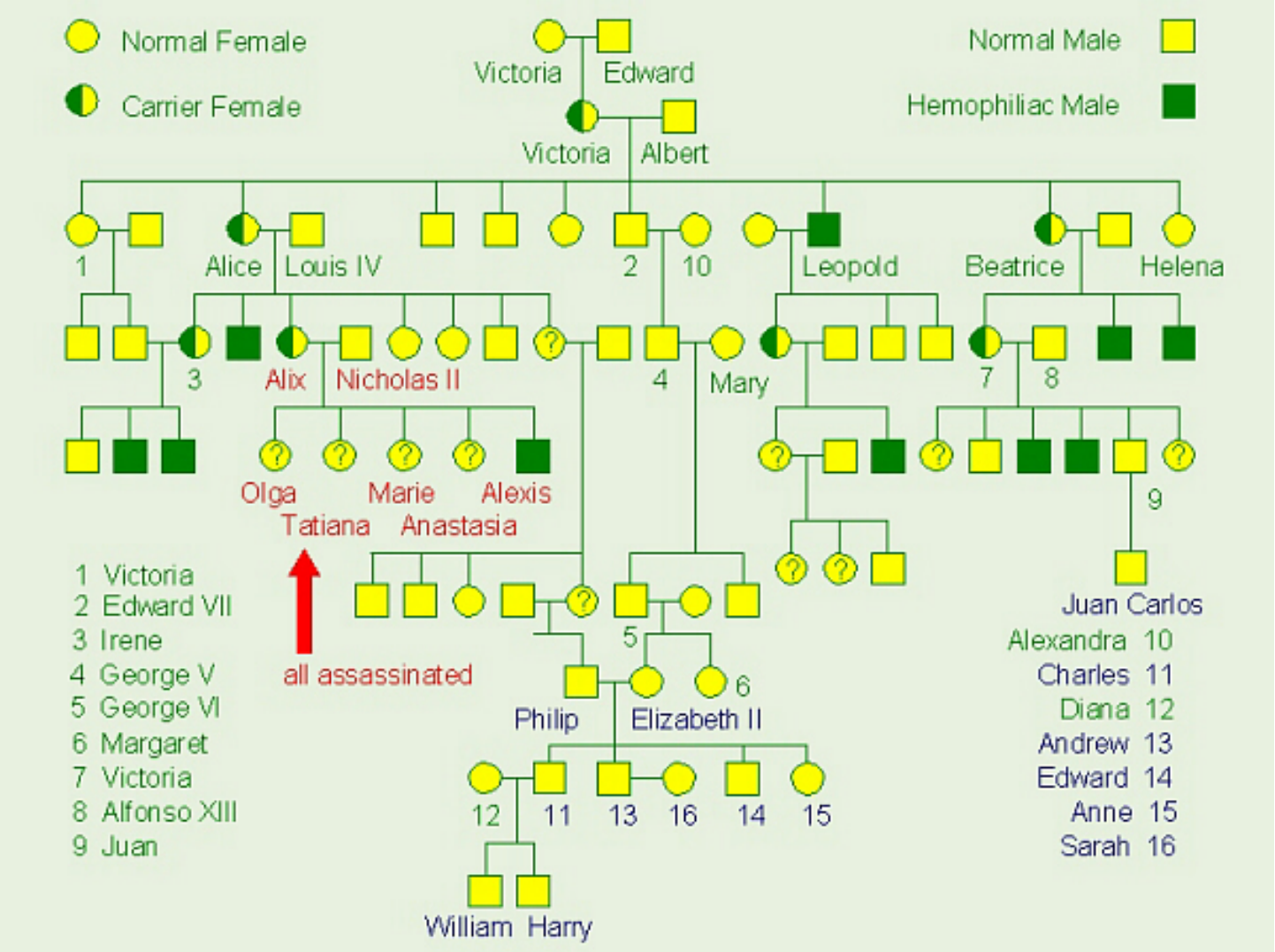
36. What was the most surprising thing that you learned from doing this assignment?

I thought it was surprising that it took so long for archeologists to find the second grave site even though it is only 70 meters away. I also really surprised that they were buried separately in the first place. Something must have happened for them to do that, but we won't find out anytime soon.

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