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Old Dominion University/BIOL294-Genetics/Rinehart-Kim/Module 9/Romanov Project

You may use various Internet sources to answer any of the questions, but please cite any sources that you use if they are not ones that I suggest.

History

You may use various Internet sources here, but please cite any sources that you use (unless I have given it to you). You will be using the following source for another section in this assignment, but it might help you answer some of the questions in this section. If you use this source to answer the following questions, you do not need to cite it.

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.)

1. **Who were the Romanov's (in Russian history)?**

   The Romanovs were the last imperial dynasty to rule in Russian history. They consisted of high-ranking aristocrats

2. **How long were the Romanovs in power in Russia?**

   The Romanovs ruled for over 300 years.

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

   Nicholas II was the Russian Tsar known as 'Tsar Nicholas II' or the 'Emperor and Autocrat of All the Russians'.

4. **Politically, what happened to Nicholas II?**

   His throne was abdicated due to the violent Bolshevik Revolution. The Bolsheviks, led by Vladimir Lenin, were against the Tsar and his rule. This led to the end of a dynasty and a rise to communism in Russia.

5. **Who took control after Nicholas II abdicated the throne?**

   The Bolsheviks, led by Lenin, took control of Russia.

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

   His family, including his wife and their five children, and four attendants, were exiled in Yekaterinburg, Russia. Thereafter, they were executed in order to break the people's loyalty to the Tsar in 1918.

7. **One of the reasons that the family of Nicholas II was executed 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 anti-Bolsheviks. The Bolsheviks were attempting to rule Russia, but did not have the support of the White Russian Army.

Hemophilia

The pedigree chart below comes from the Module powerpoint lecture notes.

8. **How was Nicholas II's wife, Alix, related to Queen Victoria of England?**

Alix was Queen Victoria's grandchild. Alix's mother, Alice, was Queen Victoria's daughter.

Both Queen Victoria and Alix are designated as being carriers for 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?)

A person suffering from Hemophilia has lower amounts of clotting factors that contribute to proteins working properly to allow blood clot. These factors are known as factor VIII (8) and factor IX (9). Factor 8 is responsible for hemophilia A, whereas factor 9 is responsible for hemophilia B. The lower the amount of either one of these factors, the more severely hemophilia affects a person. Since the blood is unable to clot, excessive bleeding occurs from injuries as small as paper cuts.

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

Use the following source for the questions 8 & 10: *http://www.ncbi.nlm.nih.gov/pubmed/20557352*
(You won't be able to access the entire article, but the abstract will give you the information you need to answer the questions.)

A hemophilia carrier is a female who has the gene that causes hemophilia A or B. Since females have XX chromosomes, they can have one working hemophilia gene and one non-working hemophilia gene. The working gene can produce sufficient amounts of clotting factors, therefore, keeping the factors at a normal range. A male cannot simply be a carrier without exhibiting symptoms because males have XY chromosomes and if the X is a mutated hemophilia gene then they will have symptoms.

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

The pedigree chart depicts Hemophilia B.

12. Using your knowledge from Module 4, on what chromosome is the gene that, when mutated, causes hemophilia?

The gene, when mutated, that causes hemophilia is on the X-chromosome.

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

Researchers, upon discovery of her tomb, were able to see a substitution in the splice acceptor site of exon 4 in the F9 gene. This mutation, itself, is considered to be the cause of hemophilia B in the royal family.

14. Using your knowledge from Module 7, describe how the mutation you described in #10 could result in a faulty gene product. Be *very* specific in your description.

Since researchers were able to identify that the mutation as a substitution we are able to better understand how a faulty gene may have been produced. A substitution is a method of mutation where a base pair is incorrectly paired with another base pair. For instance, an adenine is paired with a guanine instead of being paired with a thymine. There are three different types of substitution mutations, also referred to as point mutations, which can be silent, missense and nonsense mutations. The faulty gene most likely resulted from a spontaneous mutation in the F9

gene resulting in hemophilia B in the royal family. The chances of Hemophilia B being passed to the next generation would be determined by the x-linked recessive gene inheritance pattern. This spontaneous genetic mutation is the reason why some individuals with hemophilia have no family history of the disease.

15. 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.)

-The Romanov's son, Alexis, inherited hemophilia because his mother was a carrier.

Alexei Romanov Punnett	X^H	Y
X^H	X^HX^H	X^HY
X^h	X^HX^h	X^hY

16. 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.)

-In this punnett square, the males are the only ones that have hemophilia. This is because they are statistically more likely to acquire it from a carrier mother. They have roughly a 50% chance of acquiring it when a mother is a carrier while females have statistically less of a chance.

Romanov Punnett Square	X^H	Y
X^H	X^HX^H	X^HY
X^h	X^HX^h	X^hY

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

-Yes, it is possible for a female to inherit hemophilia, even though it is very rare due to it being an X-linked disease. For a female to inherit hemophilia, the mother would have to be a carrier for hemophilia and the father would need to be a hemophiliac as well.

18. Using a Punnett square (again, draw a table or line up the genotypes), what is the probability the daughter of a mother who is a carrier and a father who does not have the disease, will be a carrier?

-The probability of the daughter of a mother who is a carrier and a father who does not have the disease is about 1/4.

Daughter Punnett Square	X^H	Y

X^H	X^HX^H	X^HY
X^h	X^HX^h	X^hY

19. Using a Punnett square (again, draw a table or line up the genotypes), what is the probability that 4 daughters of a mother who is a carrier and a father who does not have the disease, will be a carrier?

-The probability that all 4 daughters of a mother who is a carrier and a father who does not have the disease is about 1/16.

Daughter Punnett Square	X^H	Y
X^H	X^HX^H	X^HY
X^h	X^HX^h	X^hY

20. Some historians speculate that Alexis' hemophilia condition could have led to the Russian Revolution. Explain.

-Czar Nicholas II had a sick son, Alexis. His son needed healing which led to the family seeking the assistance of Rasputin, a faith healer. Rasputin, among many other things, used his connections to influence many governmental affairs. Some historians allude to the concept that Rasputin used his connections to favor his self-interest. This reportedly made the general public weary and question those in power, which could have been a catalyst to the revolution.

Molecular Analysis of People in a Mass Grave

Use the following source to help you answer the following questions:

http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0004838 (You should be able to access the entire article.)

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

-The larger grave discovered had a total number of nine bodies. The nine bodies were the husband and wife along with their 3 daughters as well as 4 members from their staff. The smaller grave located 70 meters away discovered many years later had the two missing children's bodies.

22. When were these graves discovered?

- The larger grave was discovered in the late 1970s and an official recovery was done in 1991. The smaller grave was discovered in 2007.

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

-To confirm sex and familial relationships within the mass grave the use of Nuclear DNA testing of 5 STR markers were conducted and mtDNA testing was used to confirm maternal relationship.

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

-STR means Short tandem Repeats and these STR allele frequencies analysis is useful for the identification of an individual.

25. Mitochondrial DNA testing was also done on both Nicholas II and Alexandra. Why was information from Alexandra's, but not Nicholas', mitochondrial DNA used to identify three females as belonging to Alexandra?

-Mitochondrial DNA is inherited from the mother because of this only Alexandra information would be useful.

26. HRH Prince Philip, the Duke of Edinburgh, provided mitochondrial DNA used to identify Alexandra 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?

-Queen Elizabeth II is not related to Queen Victoria due to them not having the same maternal line of inheritance. This will result in each having different mitochondrial DNA. Prince Philip's mitochondrial DNA was used because he is a maternal relative.

27. Who was missing from the mass grave?

-The mass grave was missing two of the five children. The smaller grave had Tsarevich Alexei and one of his sisters in it.

28. 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?

-No, it would not matter if one were a male or female, they just need to be maternal relatives. The general statement that can be made is that Nicholas mitochondrial DNA will not be inherited to his children, but Alexandra mitochondrial DNA will be inherited to her children.

29. 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 16169 was identified in the mitochondrial DNA of Nicholas whereas in Duke of Fife or princess Xenia it differed due to a fixed 16169 T.

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

-The term used is Heteroplasmy

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

-The three types of DNA testing used were mitochondrial DNA, autosomal STR, and Y-STR testing.

32. Of the three types of DNA you listed in #31, which one would have been used specifically to identify Alexis?

- Of the three the Y-STR test would specifically be used to identify Alexis.

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

- Distant living relative which was a cousin was the source of DNA used to identify Alexis.

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

-It was inconclusive if the daughter was Anastasia 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).
Refer to the following source to help you answer some of the following questions:
http://www.nature.com.proxy.lib.odu.edu/ng/journal/v9/n1/pdf/ng0195-9.pdf (Please note that this is a PDF of the article and is provided via the ODU Library.)

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

-Anna Anderson was claiming to be Duchess Anastasia, the daughter of Tsar Nicholas II. A tragic event took place where everyone in the Royal family was executed but the remains of Duchess Anastasia were never identified making this claim believable. The other claim was that Anna Anderson was Franzisca Schanzkowska and this theory was in fact discovered to be true.

36. Where in the US did Anna Anderson eventually settle?

-She eventually settles in Virginia

37. Whom did she eventually marry?

-She married Jack Manahan

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

-The source of her nuclear DNA was collected from hair and bowel samples.

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

- The sources of nuclear DNA for Nicholas' and Alix's were from skeletal samples.

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

-The types of analysis that were completed on the DNA was mtDNA and STR. In addition to this, sex typing was performed using an amelogenin test.

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

-Anna's mitochondrial DNA was compared to Carl Maucher and HRH Prince Philip.

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

-The hypervariable region is a location within the nuclear DNA in which base pairs of nucleotides repeat or have substitutions.

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

-The conclusion from the mtDNA from Anna Anderson was that she was not related to the royal family and that Anna Anderson was Franziska Schanzkowska.

44. 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 the children in the Romanov family were discovered in 2007 but the identity of the sister buried with Tsarevich Alexei is still unknown.

45. What did you learn from doing this assignment? (Each person in a group should answer this question. It is not a group answer.)

Yash: I found it intriguing learning how hemophilia B ravaged the Royal Family throughout the 20th century. This triggered several questions in my head as to how this gene is no longer mutated in the royal family that is alive today and allowed me to delve deeper into this research. In addition to this, it was refreshing to see how the information we learned throughout the course can be applied to real-life examples and understand how much prior knowledge we have built.

Lauren: I knew nothing about the last Tsar of Russia. I learned what type of DNA analysis test should be performed on ancient cases such as this one. Everything I learned today was in fact new to me. It was interesting to learn about an early case of identity theft and to see how it was handled by methods of science.

Crystal: I learned just how important DNA testing is in forensic Science and just how useful these techniques are to solving crimes more efficiently. It was also interesting learning how mitochondrial DNA is inherited through each generation.

Andrea: I learned how European royal families were affected by the mutation of hemophilia. Scientists were able to obtain genetic material from the young Czar Alexis, who was affected by the disease, by combining current genetic experimentation tools and the development of biological information technology.

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/

BY PLACING MY NAME BELOW (AND INCLUDING A DATE), I HAVE REVIEWED THE ANSWERS ON THIS ASSIGNMENT, AND AGREE TO THEM.

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Lauren Ba (December 09,2020)
Andrea Ayala-Lopez (December 08, 2020)
Yash Patel (December 07, 2020)
Crystal Holland (December 09,2020)

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