DNAInteractive

Recovering the Romanovs

Teacher Pages

Introduction to the Module	The teacher is provided with an overview of the entire module. For each of the three sections of the module the following is included: an abstract, objectives, student activities
Implementing the Module	For each part of the module, the teacher is given an abstract of what their students will be exploring. It will have a list of objectives and activities that have been designed to go along with the various slides seen in the website. Each activity is described for the teacher
Instructional Approach	A 5 E Instructional model* is included as one approach to organizing a lesson. This model was developed by BSCS and is based on the constructivist philosophy of learning. However, teachers may apply whatever approach they are most comfortable using * An explanation of the 5E Instructional model can be found at the end of this chart.
Type of Activity	Simulation Interactive Guided Inquiry
Target Audience	Middle School High School Advanced Biology, Advanced Placement Biology Forensic Science
Teacher Preparation	Download the teacher guide and examine all three parts of the module
Class Time	Approximately three, 40 minutes class periods are required. Time will vary depending on the depth of the lesson.
Materials	Copy Student Activity Sheets Make sure you have access to internet computers that have MacroMedia Flash Player and Quicktime

Suggestions	• This may also be assigned for homework if internet access is not available in the classroom. • This module is an excellent opening to any unit on Human Genetics. • The module may be introduced by showing the first part of the NOVA video "Anastasia" • It is possible to customize your lesson using the Lesson Builder
Assumptions of Prior Knowledge	• Mendelian genetics • Basic cell structure
Common Misconceptions	• Sex-linked traits are inherited from the father. • A male can pass a sex-linked trait on to his offspring even though he does not show the trait.
Interdisciplinary Approach	• Social Studies • European History • Language Arts
Supplemental Materials	• Video- *Anastasia Dead or Alive?* $19.95 ISNB #1-884738-66-4 http://main.wgbh.org/wgbh/shop/wga2209.html • *Last of the Czars* Video Set $39.95. Obtainable through the Discovery Channel www.discovery.com • *Nicholas & Alexandra, The Last Imperial Family of Tsarist Russia*, From the State Hermitage Museum and the State Archive of the Russian Federation, Harry N. Abrams, Inc., Publishers, 1998 ISBN: 0-8109-3687-9
Supplemental Activities	These investigations and projects provide students at all with the opportunity to demonstrate understanding of the concepts presented in the module. Several assist students to probing more deeply into the scientific and/or social implications
Correlation to the National Science Education Standards	A matrix is provided that correlates this document to the Content Standards for both grade levels 5-8 and 9-12.

5E Instructional Model – An Explanation

Engage	Creates interest Generates curiosity Raises questions Assess prior knowledge
Explore	Encourages students to work together without direct instruction from teacher Observes and listens to students as they interact Asks probing questions to redirect students investigations when necessary Provides time for students to puzzle though problems Acts as a consultant for students
Explain	Encourages students to explain concepts and definitions in their own words Asks for justification (evidence) and clarification from students Formally provides definitions, explanations and new labels Uses students previous experiences as the basis for explaining concepts
Elaborate	Expects students to use formal labels, definitions and explanations provided previously Encourages students to apply or extend concepts and skills in new situations Reminds students of alternative explanations Refers students to existing data and evidence and asks, “What do you already know?’, “Why do you think?”
Evaluate	Observes students as they apply new concepts and skills Assesses students knowledge and/or skills Looks for evidence that students have changed their thinking or behaviors Allows students to asses their own learning and group process skills Asks open-ended questions, such as “Why do you think?”, “What evidence do you have?” “What do you know about ……?” “ How would you explain…..?”

RECOVERING THE ROMANOVS

An introduction to the module: *The Mystery of Anastasia*

Anastasia Romanov was the youngest daughter of Tsar Nicholas of Russia and his wife Tsarina Alexandra. Anastasia had three older sisters, Olga, Maria and Tatiana, and a younger brother Alexei. In 1917, the Bolsheveks led by Vladimir Lenin overthrew the Romanovs. Anastasia and her family were imprisoned in Siberia and, in July of 1918, they were brutally murdered by the Bolshevik soldiers. In order to prevent those remaining loyal to the Tsar from finding his remains, the bodies were buried in a secret location.

In the 1920's an unknown woman in a mental hospital in Germany claimed to be Anastasia Romanov. While in the hospital she adopted the name Anna Anderson. Since the 1918 massacre of the Romanov family rumors have persisted that some members of the imperial family may have survived. Could this unknown woman be Anastasia Romanov the youngest daughter of Tsar Nicholas? Anna Anderson had the same hair color, eye color, height and distinctive body markings including a deformed foot, that the Romanov princess had. Could Anastasia have escaped the brutality of the Bolshevik soldiers? Anna Anderson claimed to be Anastasia until her death in 1984.

In 1991 the remains of the royal Romanov family were exhumed in Yekaterinburg, Siberia. Portions of nine skeletons were found. Scientists used various techniques to identify the skeletal remains. They were able to identify the bodies of the Tsar and Tsarina as well as three of their children. Two skeletons were missing- Anastasia and the youngest son Alexei.

What happened to Anastasia Romanov? For most of the 20th century, this question persisted without a conclusive answer. Could she have survived the massacre that took the lives of her entire family? Or did she escape and live out her life without ever being recognized for whom she truly was? In 1994, scientists were able to used modern DNA technology to analyze the evidence and determine whether Anna Anderson was really Anastasia.

5-E Instructional Model for *Recovering the Romanovs*

Engage Part I engages student interest through a series of family photos, actual film footage, and a brief narrative describing the political climate of the times.
Explore Part II explores the question of Anna Anderson's claim that she was actually Anastasia. It also explores the ways in which pedigrees can be used to show family inheritance patterns.
Explain Part II provides students with information so that they can explain how the following concepts were important in solving the mystery Pedigrees Mitochondrial vs Nuclear DNA For enrichment activities students can go to: www.dnaftb.org .(Enter DNA From the Beginning, Go to #30). For information about the genetics of mitochondrial disease go to: www.umdf.org/mitodisease/genetics.html Sex Linked Traits (DNA From the Beginning #13 Hemophilia (www.yourgenesyourhealth.org)
Elaborate Part II allows students to access data through the Cold Spring Harbor Laboratory Biosequence Server. Students are able to analyze and compare DNA sequences from the Romanov family and Anna Anderson.
Evaluate Have students produce a storyboard or poster showing how DNA technology was used to answer the question about Anastasia. Have students do research to find out how DNA sequencing is important in medicine and forensics.

Part I: Romanov Family History

Abstract

The students are introduced to the Romanov family by way of a pedigree that shows the Tsar and Tsarina and their five children. Students will learn that the youngest son Alexei inherited the sex-linked genetic disorder hemophila. Through computer links, they can learn more about the inheritance of hemophilia. Depending on the background and focus of your class, students can be encouraged to learn more about the causes, symptoms, and treatment of the disease. A pedigree illustrates how the disorder was passed through members of the royal family.

The students are also given the opportunity to view videos showing the Romanov family in their daily activities. The history of the Russian Revolution is then discussed and related to the execution of the Tsar and his family in 1918.

Objectives: After completion of this interactive computer module students will be able to:

- Identify the members of the Romanov family
- Explain the concept of sex-linked inheritance and apply it to the disease hemophilia
- Describe the disease hemophilia and explain its symptoms and causes
- Use a Punnett square to show how Alexei inherited hemophilia from his parents
- Analyze a pedigree that demonstrates the transition of the sex-linked trait hemophilia through the Royal family
- Describe the political situation in Russia that led to the Russian revolution and the assassination of the Romanov's.

Activity 1-Slide #4

Students can visit DNA from the Beginning by clicking on the Link and going to "Sex Linked Disorder", and then clicking on the animation. It will show a pedigree of Queen Victoria and her descendents. This is a classic example of sex-linked inheritance. Students are then asked to solve Punnett square problems involving the disorder hemophilia.

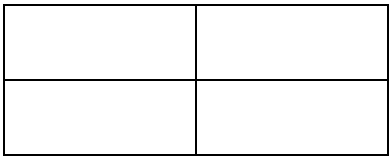
Activity 1-Slide #4

Inheritance of a Sex Linked Trait

Directions: Click on the link, sex-linked traits. Go to the animation and read through each slide until you reach the final one. This can be done by clicking on the "right arrow" just above the LINKS button. Stop when Charles Davenport introduces himself and answer the following questions:

Key: **H**=normal allele **h**=hemophilia allele, **X**=X chromosome **Y**=Y chromosome

1. Use a Punnett square to show the cross between Tsar Nicholas and Alexandra.



   a. What is the percent chance that one of their children would have the disease?

   b. What is the percent chance that only a son would have the disease?

   c. What is the percent chance that a daughter would be a carrier of the disease?

   d. How is it possible for a family with the same genotypes as the Tsar and Tsarina to have no children with hemophilia?

2. Use two different Punnett squares to show how a female can become a carrier from either her father or her mother.

Activity 1-Slide #4: Answer Key

Inheritance of a Sex Linked Trait

Key: **H**=normal allele **h**=hemophilia allele, **X**=X chromosome **Y**=Y chromosome

1. Use a Punnett square to show the cross between Tsar Nicholas and Alexandra.

	X^H	Y
X^H	$X^H X^H$	$X^H Y$
X^h	$X^H X^h$	$X^h Y$

   e. What is the percent chance that one of their children would have the disease?

      There is a 25% chance.

   f. What is the percent chance that only a son would have the disease?

      There is a 50% chance.

   g. What is the percent chance that a daughter would be a carrier of the disease?

      There is a 50% chance.

   h. How is it possible for a family with the same genotypes as the Tsar and Tsarina to have no children with hemophilia?

      The chance of having a normal child is 50%. This is true each time a child is born to the family.

2. Use two different Punnett squares to show how a female can become a carrier from either her father or her mother.

	X^H	Y
X^H	$X^H X^H$	$X^H Y$
X^h	$X^H X^h$	$X^h Y$

Carrier Mother x Normal Father

	X^h	Y
X^H	$X^H X^h$	$X^H Y$
X^H	$X^H X^H$	$X^H Y$

Normal Mother x Hemophiliac Father

Part II: The Mystery of Anna Anderson

Abstract

Students will meet the woman called Anna Anderson who claimed to be Anastasia. They will use various types of evidence to compare the two women. They will also learn more about Anna Anderson's later life when she married and moved to America.

Objectives: After completion of this interactive computer module students will be able to:

- Examine and analyze evidence that compares Anna Anderson and Anastasia
- Examine evidence such as handwriting, an ear test and facial comparison between Anna Anderson and Anastasia.
- Understand how several commonly used forensic tests are not able to provide conclusive evidence regarding Anna Anderson's claim to be Anastasia

Activity 2 – Slide #3
The Evidence: Students will use handwriting analysis, an ear test and a facial comparison chart and decide if Anna Anderson was really Anastasia.

Activity 2-Slide #3:

The Evidence

Handwriting Analysis

Follow the directions on the computer screen. Carefully examine both handwriting samples.

Do you think the same person wrote them both? _____

The Ear Test

Do the ears match? _____

In your opinion, could they be the same person? Explain your answer using evidence from the ear test.

_____
_____
_____

Face Comparison

Compare the photographs of these five different people with a photograph of the true Anastasia by rolling over each unknown face. Complete the chart by circling Yes or No for each face.

Faces	**Does this face resemble Anastasia?**	
#1	Yes	No
#2	Yes	No
#3	Yes	No
#4	Yes	No
#5	Yes	No

Write the name or names of those who most closely resemble Anastasia.

_____

Do you think that everyone making this comparison will have the same list of names that you have? ____ Explain your answer.

_____
_____
_____

Is the evidence you just finished analyzing strong enough for you to say with certainty that Anna Anderson is Anastasia. Explain your answer.

_____
_____
_____

Activity 2-Slide #3: Answer Key

The Evidence

Handwriting Analysis

Follow the directions on the computer screen. Look at both samples.

Do you think the same person wrote them both? _____

Answers will vary.

The Ear Test

Do the ears match? ***Answers will vary***.

In your opinion, could they be the same person? Support your answer with evidence from the ear test.

Students' answers will vary. However, they should provide data from the Ear Test to support their response.

Face Comparison

Complete the chart by circling Yes or No for each face.

Student answers on the chart will vary.

Write the name or names of those who most closely resemble Anastasia.

Student answers will vary.

Is the evidence you just finished analyzing strong enough for you to say with certainty that Anna Anderson is Anastasia. Explain your answer.

No, because you really can't be sure when you are comparing the handwriting, ear and face of a child with that of an adult. Not everyone will agree on the amount of similarities.

Part III: Science Solves a Mystery

Abstract

In 1991, scientists uncovered a burial site in Ykaterinburg, Siberia revealing the remains of what was believed to be the Romanov family and their staff, a total of eleven individuals. The students will view and listen to a video interview of Dr. Michael Baden who was at that time the New York City Chief Medical Examiner. He was part of an American team that was called in to examine the skeletal evidence found at the burial site.

Students will then have the opportunity to count the skeletons found at the gravesite. They will be shown ways to analyze human skeletons to determine gender and age. From this information they will determine who was missing, since only nine skeletons were present. In order to further identify the skeletons, the students are introduced to DNA technology. They first learn the difference between nuclear and mitochondrial DNA and determine who inherited the Tsarina's mitochondrial DNA by viewing a pedigree. They are then sent to a link called Sequence Server at Cold Spring Harbor Laboratory. They compare mitochondrial DNA sequences of the females found at the site with Prince Philip who is a living relative of the Tsarina. After this analysis they are able to identify the Tsarina and three of her daughters. The process is repeated using mitochondrial DNA evidence from a living relative of the Tsar. After completion of this exercise, the nine skeletons found in the grave are identified.

The final task is to determine if Anna Anderson is indeed Anastasia. By using hair and intestinal tissue samples from Anna Anderson the students are able to solve the mystery of Anastasia. The students can also view and listen to a video of Syd Mandelbaum. He directed the mitochondrial DNA analysis of Anna Anderson's hair. A second video focuses on Dr. Baden who discusses the testing of the intestinal tissue.

Objectives: After completion of this interactive computer module students will be able to:

- Compare nuclear and mitochondrial DNA
- Determine family relationships based on mitochondrial DNA
- Analyze DNA sequences to identify members of the Romanov family
- Compare mitochondrial DNA sequences to determine if Anna Anderson was Princess Anastasia or a German factory worker

Activity 3-Slide #3

The Bones - Students will view the Romanov burial site and remove each skeleton from the grave. This will allow them to count the actual number of skeletons present.

Activity 4-Slide #5

Skeletal Analysis - Each of the nine skeletons will be analyzed into order to determine its age and sex. Wisdom teeth, vertebrae, and pelvic bones are the key skeletal structures students will examine during this activity.

Activity 5-Slide #8

The DNA - Students are given a brief tutorial on nuclear vs. mitochondrial DNA. They are then asked to apply what they have learned to the transfer of mitochondrial DNA through the Romanov family.

Activity 6-Slide #12-13

The Tsarina's Pedigree/Analyzing the DNA - Students will determine the closest living relative of the Tsarina by tracing mitochondrial DNA through her family pedigree.

Students will then learn how to use the Bioservers' Sequence Server at Cold Spring Harbor Laboratory to compare the DNA of the female skeletons found at the gravesite with a living maternal relative. This will help them to determine which females in the grave were related and therefore part of the royal family.

Activity 7-Slide #15

Students examine the Tsar's pedigree in order to identify his closest living maternal relative. The Sequence Server will again be used to compare the mtDNA of the male skeletons in order to identify the Tsar.

Activity 8-Slide #8

Students use the information they have collected to more definitively solve the mystery of Anna Anderson. They again use the Sequence Server to compare the mtDNA of Anna to a person thought to be her maternal.

ACTIVITY 3-Slide #3

The Bones

When the Soviet Empire collapsed in 1989, many long held secrets were finally revealed. Among them, was the secret burial location of Czar Nicholas and his family. In 1992, a group of scientists uncovered their remains and made some startling discoveries. Could these bones once and for all solve the great mystery of Anastasia?

Killed along with the royal family were two male servants, the family doctor and a nurse. Altogether, eleven people were murdered that night in Siberia: 6 females and 5 males. The following chart lists who they were and the ages of the children.

Females	**Males**
Tsarina Alexandra, adult	Tsar Nicholas, adult
Princess Olga, 22 years old	Prince Alexei, almost 14 years old
Princess Tatiana, 21 years old	Family doctor, adult
Princess Maria. 19 years old	Servant, adult
Princess Anastasia, 17 years old	Servant, adult
Nurse, adult	

How many skeletons do we expect to find? _____

Click on any bone in the grave to remove a skeleton. Continue until no bones remain in the grave.

How many skeletons were found in the grave?_____

ACTIVITY 3-Slide #3: Answer Key

The Bones

How many skeletons do we expect to find? ***11***

How many skeletons were found in the grave? ***9***

ACTIVITY 4-Slide #5

Skeletal Analysis

Click on the "Analyze the Skeletons" box and then on skeleton #1.

Analyze the bones by rolling over the boxes. Use the key to the right of the screen to determine if the wisdom teeth are present, if rings on the vertebrae are present and if the pelvis is that of a male or of a female. Then circle the correct choices in the chart below.

Do the same for the other 8 skeletons. Use this information and the list from the previous activity of who was murdered to determine whose skeleton is present and whose is missing.

Skeletons	#1	#2	#3	#4	#5	#6	#7	#8	#9
Wisdom teeth present? Yes = 22 years and older	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	No	No	No	No	No	No	No	No	No
Rings on vertebrae? (Yes = 18 years and older.)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	No	No	No	No	No	No	No	No	No
Pelvis- male or female?	Male	Male	Male	Male	Male	Male	Male	Male	Male
	Female	Female	Female	Female	Female	Female	Female	Female	Female
Give a possible identity for each skeleton									
Who is missing?									

ACTIVITY 4-Slide #5: Answer Key

Skeletal Analysis

Skeletons	#1	#2	#3	#4	#5	#6	#7	#8	#9
Wisdom teeth present? Yes = 22 years and older	***Yes***	***Yes***	Yes	***Yes***	***Yes***	Yes	***Yes***	***Yes***	***Yes***
	No	No	***No***	No	No	***No***	No	No	No
Rings on vertebrae? (Yes = 18 years and older.)	***Yes***	***Yes***	***Yes***	***Yes***	***Yes***	***Yes***	***Yes***	***Yes***	***Yes***
	No	No	No	No	No	No	No	No	No
Pelvis- male or female?	***Male***	***Male***	Male	***Male***	Male	Male	Male	***Male***	Male
	Female	Female	***Female***	Female	***Female***	***Female***	***Female***	Female	***Female***
Give a possible identity for each skeleton	Tsar	Family Doctor	Princess Maria	Servant	Tsarina	Princess Tatiana	Nurse	Servant	Princess Olga
Who is missing? ***Anastasia and Alexei***									

Activity 5-Slides #8 - #11

The DNA

Although historical evidence tells us that these are the remains of the murdered royal family, how can the identity of these bones be proven?

_____
_____
_____
_____

Because there are no direct descendents of the Romanov family and the only surviving relatives are distant, the best way to determine the identity of the skeletons is to use DNA from the mitochondria. (Refer to slide #8 for background information)

Mitochondria are organelles that contain a small circular chromosome. Only the mother passes on her mitochondrial DNA to the children in a family (both her sons and daughters). Mitochondrial DNA remains relatively unchanged for many generations.

Do you have mitochondrial DNA? _____ If so, who gave it to you? _____

Try "Test Yourself on Mitochondrial DNA" on Slide #11

From whom did the Romanov children receive their mitochondrial DNA (mtDNA)?

_____

Where did that the person who passed their mtDNA on to the Romanov children get their mtDNA? _____

Does Tsar Nicholas II have the same mtDNA as his children? _____ Support your answer with an explanation of what you know about mitochondrial DNA.

_____
_____
_____

How can the identity of the skeletal remains be proven?

_____
_____
_____
_____

Activity 5-Slides #8 - #11: Answer Key

The DNA

Although historical evidence tells us that these are the remains of the murdered royal family, how can the identity of these bones be proven?

The remains can be identified through DNA analysis.

Do you have mitochondrial DNA? ***Yes*** If so, who gave it to you? ***My mother***

From whom did the Romanov children receive their mitochondrial DNA (mtDNA)?

They received their mtDNA from their mother, the Tsarina Alexandra

Where did that person who passed their mtDNA on to the Romanov children get their mtDNA?

This individual (the Tsarina) received her mitochondrial DNA from her mother..

Does Tsar Nicholas II have the same mtDNA as his children? ***No*** Support your answer with an explanation of what you know about mitochondrial DNA.

Tsar Nicholas received his mtDNA from his mother. Mitochondrial DNA is passed from the mother on to all of her offspring. The father does not contribute any mtDNA to the children.

How can the identity of the skeletal remains be proven?

The identity can be proven by comparing the mtDNA of the skeletal remains to that of the maternal relatives of the Tsarina and the Tsar. The wisdom teeth, vertebrae, and pelvic structure can also be used since mtDNA does not provide any information about the age or the sex of an individual.

Activity 6-Slides #12 - #13

The Tsarina's Pedigree - Analyzing the DNA

Click on the Tsarina Alexandra's pedigree.

What do the small red objects represent? _____

After examining the Tsarina's pedigree, record the name of the most recent living Romanov maternal relative. _____

How can this maternal relative aid scientists in confirming that the skeletal remains belong to the Romanov family? _____

_____

_____

Go to Slide 13

Your next step is to confirm the identity of the skeletons found in the grave starting with the females.

How many of the females should be related to each other? _____

Should those that are related to one another have the same mitochondrial DNA (mtDNA)? _____ Explain your answer _____

_____

Click on the Bioservers' Sequence Server

Which sequence does not match the others?_____ Therefore, which of the females skeletons are related to one another? _____

Go to Slide 14

Based on the Sequence Server results that compared the mtDNA of the skeletons to the mtDNA of Prince Philip, answer the following questions.

1. What can we conclude about the skeletons?_____
2. What can we conclude about skeleton #9?_____

Activity 6-Slides #12 - #14: Answer Key

The Tsarina's Pedigree - Analyzing the DNA

What do the small red objects represent? ***Mitochondria***

After examining the Tsarina's pedigree, record the name of the most recent living Romanov maternal relative. ***Prince Philip of England, Duke of Edinburgh***

How can this maternal relative aid scientists in confirming that the skeletal remains belong to the Romanov family?

This person would have the same mtDNA as the Tsarina and her children.

How many of the females should be related to each other? ***4***

Should those that are related to one another have the same mitochondrial DNA (mtDNA)? ***Yes*** Explain your answer. ***All of them would have received their mtDNA from the Tsarina. This same mtDNA was p[assed along through the family from mother to children ending in Prince Philip.***

What can we conclude about the skeletons?

Skeletons # 3,5,6 and 7 are related to Prince Philip

What can we conclude about skeleton #9?

This skeleton is not related to the royal family

Activity 7-Slide # 15-16

The Tsar's Pedigree
Analyzing the DNA

Click on the Tsar's Pedigree.

Is there a maternal relative alive that can be used for a mitochondrial DNA comparison?

If so, who? _____

Now you must determine which male skeleton is the Tsar.

Click on Slide #16. Go the Bioservers' Sequence Server. The differences will again be highlighted in yellow.

Click "Compare." The differences will again be highlighted in yellow.

Is there a skeleton that matches the Tsar's family? _____

If so, which number skeleton is it? _____

What conclusion can be made about the male skeletons? _____

_____

_____

_____

Activity 7-Slide # 15-16: Answer Key

The Tsar's Pedigree
Analyzing the DNA

Is there a maternal relative alive that can be used for a mitochondrial DNA comparison?

If so, who? ***Countess Xena Cheremeteff***

Is there a skeleton that matches the Tsar's family? ***Yes.***

If so, which number skeleton is it? ***It is skeleton number 4.***

What conclusions can be made about the male skeletons?

One of the skeletons is the Tsar. The other male skeletons are not relatives.

Activity 8 – Slides #18 - #19

What About Anna Anderson?

The same mitochondrial DNA technology could be used to reveal Anna Anderson's true identity? Explain how this could be done.

_____

_____

_____

Who would you expect to match Anna Anderson's mitochondrial DNA sequence if she is really Anastasia? _____

Go to the Sequence Server and compare Anna Anderson's mtDNA with that of Prince Philip and Carl Maucher.

What do the findings suggest? _____

_____

_____

Is Anna Anderson really Anastasia? _____

Activity 8 – Slides #18 - #19: Answer Key

What About Anna Anderson?

The same mitochondrial DNA technology can be used to reveal Anna Anderson's true identity. Explain how this could be done.

Scientists could examine her mtDNA and see if it matches the mtDNA of the Tsarina's maternal family line.

Who's mtDNA sequence would you expect to match Anna Anderson's mtDNA sequence if she is really Anastasia?

She should match the mtDNA sequence of the Tsarina and Prince Philip.

What do the findings suggest?

The results indicate that Anna Anderson's is not related to Prince Philip and the Tsarina but is related to Carl Maucher.

Is Anna Anderson really Anastasia? ***No, she is not.***

Enrichment Activities

Middle School

- Draw a pedigree of your own family. Start with your maternal and paternal grandparents. Include aunts, uncles, and cousins. If possible add great-grandparents.
- Write an ending to the story of Anastasia. Write it as if Anna Anderson did indeed prove to be Anastasia.
- What if someone claimed to be Anastasia's brother Alexei? Explain how you would go about determining whether or not this person really is Alexei.
- Who was Rasputin and how is he part of the Romanov story?

High School

- Research Vladimir Lenin, the Bolsheviks and the Russian Revolution. Create a historical timeline.
- Research Rasputin, the notorious monk who was very much a part of the story of the Romanov's.
- What if someone claimed to be Anastasia's brother Alexei? Are there any individuals present in either the Tsar's or Tsarina's family tree you could use to make a decision regarding the claim. If so, who are these individuals? If not, why is there no one?
- Do further research to find out about other similarities between Anna Anderson and the real Anastasia. Find out who believed in her and who didn't.
- After sequencing the mitochondrial DNA of the Tsar, scientists discovered another mystery. It seems that the Tsar had two types of mitochondrial DNA. One type contained a C (cytosine) at position 16169 while the other type contained a T (thymine) at the same location. His relatives only had thymine. On the following page, read the article "Calibrating the Mitochondrial Clock." (Science, Volume 279, 1/2/98) and answer the following questions.
  1) What is the scientific term used to describe the anomaly discovered in the Tsar's mt DNA?
  2) Why did this finding lead to controversy over the authenticity of the skeletons found in the Siberian grave?
  3) How was the question of the Tsar's bones eventually resolved?
  4) Explain how the mtDNA mutation rate is used as a clock to date key events in human history.
  5) Why has this new finding resulted in concern about the mutation rate used by scientists in the mitochondrial clock?
  6) How might heteroplasmy get a criminal off the hook?

Calibrating the Mitochondrial Clock

Ann Gibbons

Mitochondrial DNA appears to mutate much faster than expected, prompting new DNA forensics procedures and raising troubling questions about the dating of evolutionary events

In 1991, Russians exhumed a Siberian grave containing nine skeletons thought to be the remains of the last Russian tsar, Nicholas II, and his family and retinue, who were shot by firing squad in 1918. But two bodies were missing, so no one could be absolutely certain of the identity of the remains. And DNA testing done in 1992--expected to settle the issue quickly--instead raised a new mystery.

Some of the DNA from the tsar's mitochondria--cellular organelles with their own DNA--didn't quite match that of his living relatives. Forensic experts thought that most people carry only one type of mitochondrial DNA(mtDNA), but the tsar had two: The same site sometimes contained a cytosine and sometimes a thymine. His relatives had only thymine, a mismatch that fueled controversy over the authenticity of the skeletons.

The question of the tsar's bones was finally put to rest after the remains of his brother, the Grand Duke of Russia Georgij Romanov, were exhumed; the results of the DNA analysis were published in *Nature Genetics* in 1996. Like the tsar, the duke had inherited two different sequences of mtDNA from their mother, a condition known as heteroplasmy. But solving the mystery of the Romanov's remains raised another puzzle that first troubled forensics experts and is now worrying evolutionists. "How often will this heteroplasmy pop up?" wondered Thomas J. Parsons, a molecular geneticist at the Armed Forces DNA Identification Laboratory in Rockville, Maryland, who helped identify the tsar's bones.

Several new studies suggest that heteroplasmy may in fact be a frequent event. They have found that it occurs in at least 10% and probably 20% of humans, says molecular biologist Mitchell Holland, director of the Armed Forces lab. And because heteroplasmy is caused by mutations, this unexpectedly high incidence suggests that mtDNA mutates much more often than previously estimated--as much as 20-fold faster, according to two studies that are causing a stir. Other studies have not found such rapid mutation rates, however.

Resolving the issue is vital. For forensic scientists like Parsons, who use mtDNA to identify soldiers' remains and to convict or exonerate suspects, a high mutation rate might cause them to miss a match in their samples. It could also complicate the lives of evolutionary scientists who use the mtDNA mutation rate as a clock to date such key events as when human ancestors spread around the globe.

Evolutionists have assumed that the clock is constant, ticking off mutations every 6000 to 12,000 years or so. But if the clock ticks faster or at different rates at different times, some of the spectacular results--such as dating our ancestors' first journeys into Europe at about 40,000 years ago--may be in question. "We've been treating this like a stopwatch, and I'm concerned that it's as precise as a sun dial," says Neil Howell, a geneticist at the University of Texas Medical Branch in Galveston. "I don't mean to be inflammatory, but I'm concerned that we're pushing this system more than we should."

Counting mutations

The small circles of DNA in mitochondria have been the favored tool for evolutionary and forensic studies since their sequence was unraveled in 1981. Unlike the DNA in the nucleus of the cell, which comes from both egg and sperm, an organism's mtDNA comes only from the mother's egg. Thus mtDNA can be used to trace maternal

ancestry without the complicating effects of the mixing of genes from both parents. And every cell in the body has hundreds of energy-producing mitochondria, so it's far easier to retrieve mtDNA than nuclear DNA.

It seemed like a relatively straightforward genetic system. Researchers could count the differences in the same sequence of mtDNA in different groups of people and, assuming a constant mutation rate, calculate how long ago the populations diverged. But the case of the tsar highlights how little is known about the way mtDNA is inherited. His mother must have carried or acquired a mutation, so there were hundreds of copies of each of two kinds of mtDNA in her egg cells. She then passed some of each kind to her sons. But just how often do such mutations occur?

The most widely used mutation rate for noncoding human mtDNA relies on estimates of the date when humans and chimpanzees shared a common ancestor, taken to be 5 million years ago. That date is based on counting the mtDNA and protein differences between all the great apes and timing their divergence using dates from fossils of one great ape's ancestor. In humans, this yields a rate of about one mutation every 300 to 600 generations, or one every 6000 to 12,000 years (assuming a generation is 20 years), says molecular anthropologist Mark Stoneking of Pennsylvania State University in University Park. Those estimates are also calibrated with other archaeological dates, but nonetheless yield wide margins of error in published dates. But a few studies have begun to suggest that the actual rates are much faster, prompting researchers to think twice about the mtDNA clock they depend upon.

For example, after working on the tsar's DNA, Parsons was surprised to find heteroplasmy popping up more frequently than expected in the families of missing soldiers. He and his colleagues in the United States and England began a systematic study of mtDNA from soldiers' families and Amish and British families. Like most such studies, this one compares so-called "noncoding" sequences of the control region of mtDNA, which do not code for gene products and therefore are thought to be free from natural selection.

The researchers sequenced 610 base pairs of the mtDNA control region in 357 individuals from 134 different families, representing 327 generational events, or times that mothers passed on mtDNA to their offspring. Evolutionary studies led them to expect about one mutation in 600 generations (one every 12,000 years). So they were "stunned" to find 10 base-pair changes, which gave them a rate of one mutation every 40 generations, or one every 800 years. The data were published last year in *Nature Genetics,* and the rate has held up as the number of families has doubled, Parsons told scientists who gathered at a recent international workshop* on the problem of mtDNA mutation rates.

Howell's team independently arrived at a similar conclusion after looking deep within the pedigree of one Australian family affected with Leber hereditary optic neuropathy, a disease caused by an mtDNA gene mutation. When the researchers analyzed mtDNA from 40 members of this family, they found that one individual carried two mutations in the control region (presumably unrelated to the disease, because it is noncoding mtDNA). That condition is known as triplasmy, because including the nonmutated sequence, he had three different mtDNA sequences in his cells.

By tracing the mutations back through the family pedigree, Howell was able to estimate that both mutations probably arose in the same woman who was born in 1861, yielding an overall divergence rate of one mutation every 25 to 40 generations. "Both of our studies came to a remarkably similar conclusion," says Howell, whose study was published in late 1996 in the *American Journal of Human Genetics*. Both also warned that phylogenetic studies have "substantially underestimated the rate of mtDNA divergence."

Several teams of evolutionists promptly went back to their labs to count mtDNA mutations in families of known pedigree. So far, Stoneking's team has sequenced

segments of the control region in closely related families on the Atlantic island of Tristan da Cunha, where pedigrees trace back to five female founders in the early 19th century. But neither that study nor one of 33 Swedish families has found a higher mutation rate. "After we read Howell's study, we looked in vain for mutations in our families," says geneticist Ulf Gyllensten of Uppsala University in Sweden, whose results are in press in *Nature Genetics*. More work is under way in Polynesia, Israel, and Europe.

Troubled by the discrepancy in their results, the scientists have pooled their data with a few other studies showing heteroplasmy, hoping to glean a more accurate estimate of the overall mutation rate. According to papers in press by Parsons, and Stoneking and Gyllensten, the combined mutation rate--one mutation per 1200 years--is still higher than the one mutation per 6000 to 12,000 years estimated by evolutionists, although not as fast as the rate observed by Parsons and Howell. "The fact that we see such relatively large differences among studies indicates that we have some unknown variable which is causing this," says Gyllensten.

Because few studies have been done, the discrepancy in rates could simply be a statistical artifact, in which case it should vanish as sample sizes grow larger, notes Eric Shoubridge, a molecular geneticist at the Montreal Neurological Institute. Another possibility is that the rate is higher in some sites of the DNA than others—so called "hot spots." Indeed, almost all the mutations detected in Parsons and Howell's studies occur at known hot spots, says University of Munich molecular geneticist Svante Pääbo.

Also, the time span of observation plays a role. For example, because hot spots mutate so frequently, over tens of thousands of years they can revert back to their original sequences, overwriting previous mutations at that site.
As a result, the long-term mutation rate would underestimate how often hot spots mutate--and the average long-term mutation rate for the entire control region would be slower than that from near-term studies of families.
"The easiest explanation is that these two rates are caused by hot spots," says Pääbo.

If so, these short-term rates need not perturb long-term studies. "It may be that the faster rate works on the short time scale and that you use the phylogenetic rate for long term events," says Shoubridge.

But Parsons doubts that hot spots account for all the mutations he has observed. He says that some of the difference between the long-term and short-term rates could be explained if the noncoding DNA in the control region is not entirely immune to selection pressure. The control region, for example, promotes replication and transcription of mtDNA, so any mutation that interferes with the efficiency of these processes might be deleterious and therefore selected against, reducing the apparent mutation rate.

Regardless of the cause, evolutionists are most concerned about the effect of a faster mutation rate. For example, researchers have calculated that "mitochondrial Eve"--the woman whose mtDNA was ancestral to that in all living people--lived 100,000 to 200,000 years ago in Africa. Using the new clock, she would be a mere 6000 years old.

No one thinks that's the case, but at what point should models switch from one mtDNA time zone to the other? "I'm worried that people who are looking at very recent events, such as the peopling of Europe, are ignoring this problem," says Laurent Excoffier, a population geneticist at the University of Geneva. Indeed, the mysterious and sudden expansion of modern humans into Europe and other parts of the globe, which other genetic evidence puts at about 40,000 years ago, may actually have happened 10,000 to 20,000 years ago--around the time of agriculture, says Excoffier. And mtDNA studies now date the peopling of the Americas at 34,000 years ago, even though the oldest noncontroversial archaeological sites are 12,500 years old. Recalibrating the mtDNA clock would narrow the difference (*Science,* 28 February 1997, p. 1256).

But not everyone is ready to redate evolutionary history on the basis of a few studies of mutation rates in living people. "This is all a fuss about nothing," says Oxford University geneticist Martin Richards, who thinks the fast rate reaches back hundreds of years at most.

That, however, is squarely within the time frame of forensics cases. Heteroplasmy isn't always a complicating factor in such analyses. When it exists in more than one family member, the confidence in the identification gets stronger, as in the case of the tsar. But otherwise, it could let a criminal off the hook if his mtDNA differed by one nucleotide from a crime scene sample. Therefore, Parsons and Holland, in their work identifying 220 soldiers' remains from World War II to the present, now have new guidelines--adopted by the FBI as well—to account for a faster mutation rate. When a missing soldier's or criminal suspect's mtDNA comes up with a single difference from that of a relative or at a crime scene, the scientists no longer call it a "mismatch." Instead the results are considered "inconclusive." And, for now, so are some of the evolutionary results gained by using the
mtDNA clock.

* First International Workshop on Human Mitochondrial DNA, 25 to 28 October 1997, Washington, D.C.

Other Websites and Activities Featuring
Anastasia Romanov

General Interest

Clicking Anastasia: An educational Internet adventure where players search for the lost Romanov fortune. http://www.lostsecrets.com

AP Biology

Hint: Check out this Link! www.yourgenesyourhealth.org It contains a wealth of information!

Have your AP students find the answers to the following questions:

1) About 20% of males with hemophilia get the disease even their mother does not carry the mutated gene. How is this possible?

2) Explain the difference between mild and severe cases of hemophilia. Base your answer on the different types of mutations that cause the disease.

3) Explain how some female carriers show a mild form of hemophilia resulting from X-inactivation.

The Content Standards	Correlation to *Recovering the Romanovs*
Content Standard A: As a result of activities in grades 5-8 all students should develop the abilities necessary to do and understand scientific Inquiry	
▪ Identify questions that can be answered through scientific investigations	Activities 2,3,6,7,8
▪ Use appropriate tools and techniques to gather, analyze, and interpret data	Activities 1,2,3,4,6,7,8
▪ Develop descriptions, explanations, predictions, and models using evidence	Activities 2,4,5,6,7,8
▪ Think critically and logically to make the relationships between evidence and explanations	Activities 1,2,4,6,7,8
▪ Recognize and analyze alternative explanations and predictions	Activities 1,2,8
Content Standard C: Life Science **As a result of their activities in grades 5-8, all students should develop understanding of reproductions and heredity**	
▪ Organism require a set of instructions for specifying its traits	Activity 1
▪ Hereditary information is contained in genes and can be passed from one generation to another	Activities 1,5,6,7
▪ Characteristics of organisms can be described in terms of combinations of traits	Activity 1
Content Standards Grades 9-12	
Content Standard A: As a result of activities in grades 9-12, all students should develop Abilities necessary to do and understand scientific inquiry	
▪ Identify questions and concepts that guide scientific investigations	Activities 2,3
▪ Recognize and analyze alternative explanations and models	Activities 1,2
Content Standard C: Life Science **As a result of their activities in grades 9-12, all students should develop an understanding of the molecular basis of heredity**	
▪ In all organisms, the instructions for specifying characteristics of the organism are carried in the DNA	Activities 1,5,6,7,8
▪ Variations that are hidden in one generation can be expressed in the next	Activity 1
▪ Changes in DNA (mutations) occur spontaneously at low rates	Activity 1
Content Standard G: As a result of activities in grades 9-12, all students should develop and understanding of science as a human endeavor, the nature of scientific knowledge, and historical perspectives.	
• Science as a human endeavor	Activities 3,4,5,6,7,8