Identification of the Romanov family by DNA Analysis

Jules Brockerville and Clare Youden

History

- 1917: Bolshevik revolution
- 1918: Family in custody, then murdered
- Government denied they had been killed until 1926

History

(Romanov-Memorial, 1999)

- 1979: Geologist, Dr. Alexander Avdonin, discovered grave location
- 1991: Soviet union falls and grave location is released, official excavation took place

History

(Romanov-Memorial, 1999)

- 9 Individuals in grave
- Anthropological analysis suggests Tsarevich Alexei and one sister missing

Anthropological Analysis

Skeleton #	Sex	Age	Size	Russian team conclusion	American team conclusion
5	F	~20	166-171	Tatiana	Maria
6	F	~20	162-171	Anastasia	Tatiana
Bodies not present ->				Alexei and Maria	Alexei and Anastasia

(Modified from Romanov-Memorial, 1999)

The Lost Sister

- Mystery surrounding “missing” sister
- **Anastasia** most commonly depicted
- “Anastasia” (1997) movie
- Anna Anderson (right) Anastasia impostor

Researchers

- Dr. Peter Gill
- Pioneer in forensic DNA
- Team from UK to identify remains

- Dr. Pavel Ivanov (left)
- Russian Scientist

- Dr. Erika Hagelberg
- Selected to independently confirm findings

Sample processing

(Durham University)

- Hagelberg & Clegg method
- Outer surfaces of bone removed
- Remaining bone frozen and ground into fine powder
- DNA extracted with ethanol, chloroform, centrifuged
- DNA extracts quantified by hybridization

Laboratory conditions

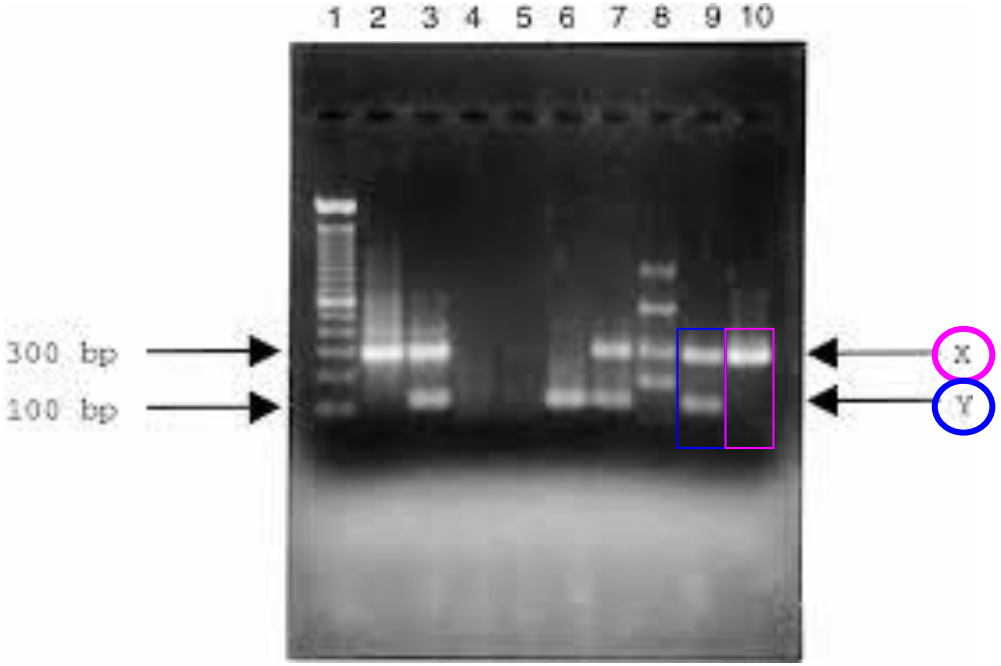
(Research Gate)

- All extractions took place in laminar flow cabinet in dedicated laboratory
- Amplification done in different laboratory
- Negative controls and duplicates

Goals of analysis

1. Establish sexes of remains
2. Establish skeletons to family group
3. Establish as Romanovs

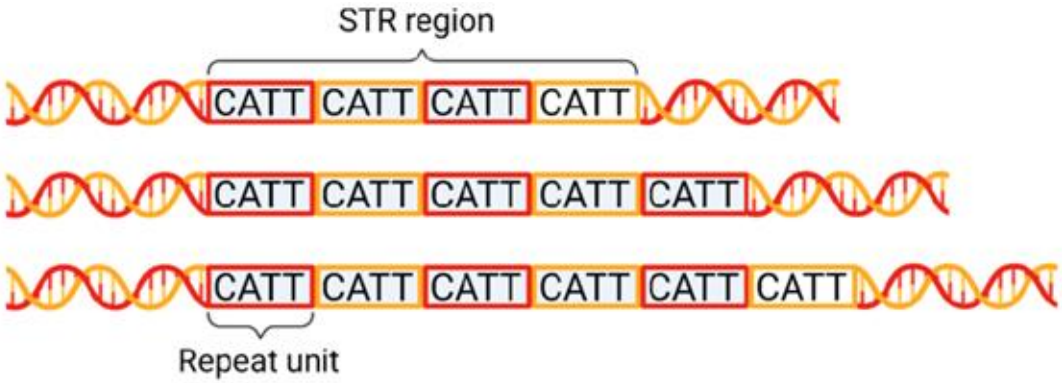
Sex testing



(Stewart et al., 2016)

- Amplified portion of X-Y homologous gene (amelogenin)
- X and Y specific products generated from single primer pair
- XX: one band, XY: two bands
- Found four males and five females

Establishing relatedness



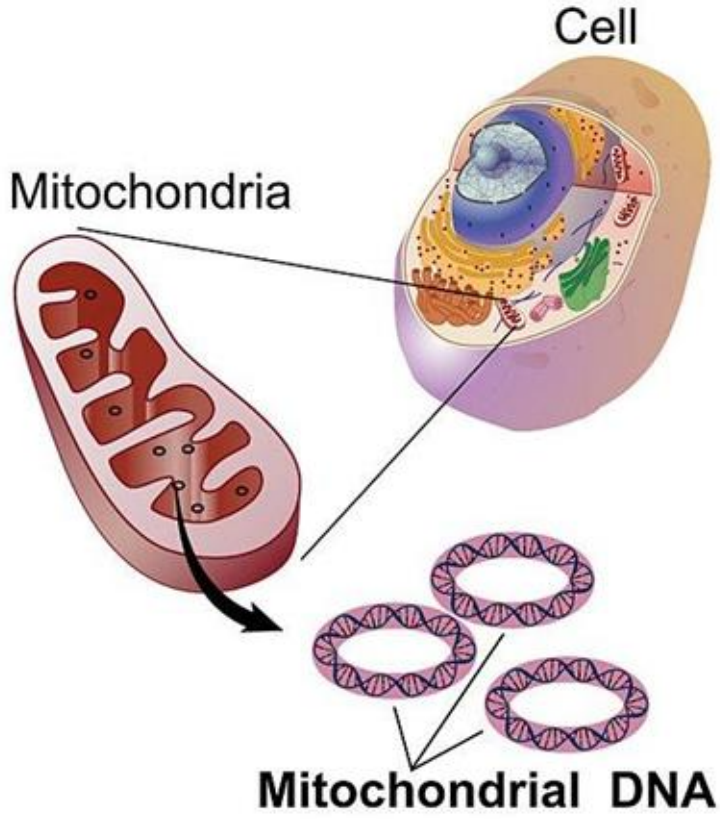
- Used **Short Tandem Repeats (STRs)**
- Number of times DNA sequence repeated at STR locus differs between individuals
- Used PCR to analyze 5 loci in each skeleton
  - Cycled 35-43 times, depending on loci

Establishing relatedness

Table 1 STR genotypes[a] for the nine skeletons

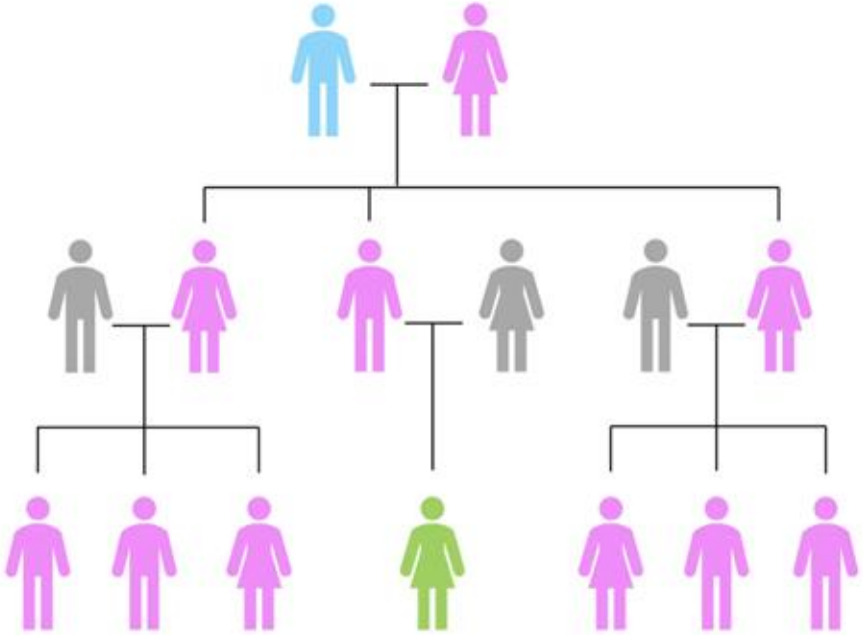
Skeleton	HUMVWA/31	HUMTH01	HUMF13A1	HUMFES/FPS	HUMACTBP2
1 (servant)	14,20	9,10	6,16	10,11	ND
2 (doctor)	17,17	6,10	5,7	10,11	11,30
3 (child)	15,16	8,10	5,7	12,13	11,32
4 (Tsar)	15,16	7,10	7,7	12,12	11,32
5 (child)	15,16	7,8	5,7	12,13	11,36
6 (child)	15,16	8,10	3,7	12,13	32,36
7 (Tsarina)	15,16	8,8	3,5	12,13	32,36
8 (servant)	15,17	6,9	5,7	8,10	ND
9 (servant)	16,17	6,6	6,7	11,12	ND

Mitochondrial DNA



- Multiple copies per cell, one copy nuclear DNA
- Circular
- Test snippet of circular DNA
- Has 2 hypervariable regions (HV1 and HV2)
- “Lineage marker”

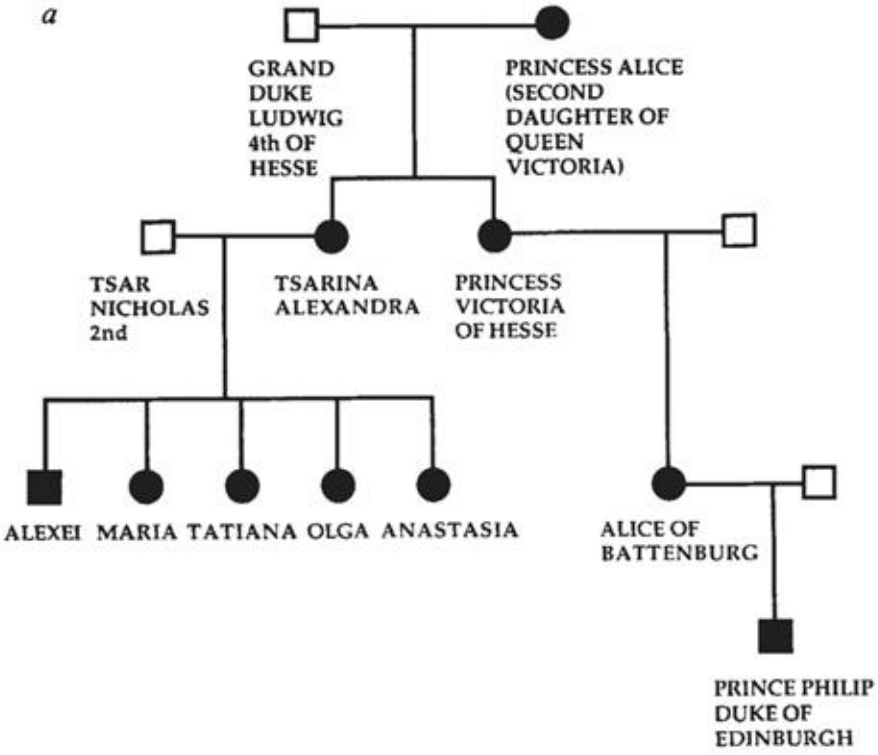
Mitochondrial DNA



(Karen Cummings, 2024)

- Multiple mtDNA genomes, one nuclear DNA genome
- Circular
- Test snippet of circular DNA
- Has 2 hypervariable regions (HV1 and HV2)
- **“Lineage marker”**

Establishing family



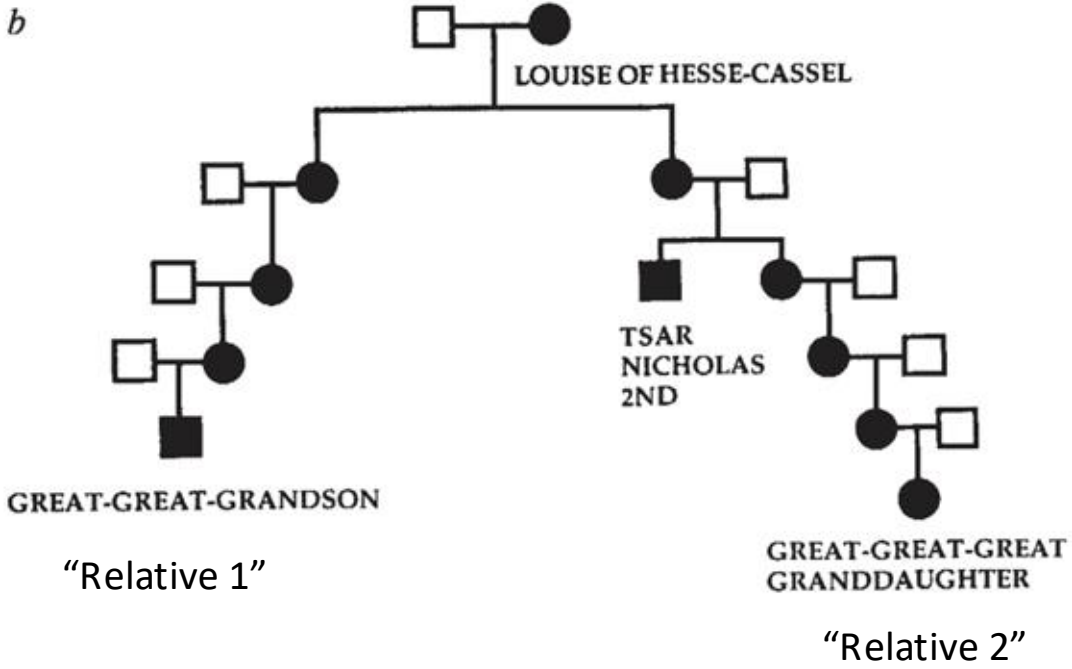
- **Prince Philip** grand-nephew of unbroken maternal descent of Tsarina Alexandra
- Consents to give blood
- Confirm mother and sibling status of children

Establishing family

Table 2 Summary of mtDNA differences compared to the Anderson[21] reference sequence

Origin of sample	DNA source	Length sequenced (bp)	Positions within hypervariable regions (HVR) of mitochondrial DNA																			
			HVR 1												HVR 2							
			16111	16126	16169	16261	16264	16278	16293	16294	16296	16304	16311	16357	73	146	195	263	309.1	309.2	315.1
			C	T	C	C	C	C	A	C	C	T	T	T	A	T	T	A	*	*	*
Servant 1 (?)	Femur skeleton 1	760	.	.	.	.	.	.	.	.	.	C	.	.	–	.	.	G	C	C	C
Servant 2 (?)	Femur skeleton 8	742	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	G	.	.	C
Servant 3 (?)	Femur skeleton 9	650	.	.	.	T	.	T	G	.	.	.	C	.	–	.	C	G	.	.	C
Royal Physician Dr Botkin (?)	Femur skeleton 2	736	.	.	.	.	T	.	.	.	.	.	.	.	.	C	.	G	C	C	C
Daughter 1 of Tsar/Tsarina (?)	Femur skeleton 3	755	T	.	.	.	.	.	.	.	.	.	.	C	.	.	.	G	.	.	C
Daughter 2 of Tsar/Tsarina (?)	Femur skeleton 5	634	T	.	.	.	.	.	.	.	.	.	.	C	.	.	–	G	.	.	C
Daughter 3 of Tsar/Tsarina (?)	Femur skeleton 6	760	T	.	.	.	.	.	.	.	.	.	.	C	.	.	.	G	.	.	C
Tsarina Alexandra (?)	Femur skeleton 7	744	T	.	.	.	.	.	.	.	.	.	.	C	.	.	.	G	.	.	C
Duke of Edinburgh (Grand nephew of Tsarina)	Blood sample	760	T	.	.	.	.	.	.	.	.	.	.	C	.	.	.	G	.	.	C
Tsar Nicholas II (?)	Femur skeleton 4	782	.	C	Y	.	.	.	.	T	T	.	.	.	G	.	.	G	.	.	C
Gt. Gt. grandson of Louise of Hesse-Cassel	Blood sample	781	.	C	T	.	.	.	.	T	T	.	.	.	G	.	.	G	.	.	C
Gt. Gt. Gt. granddaughter of Louise of Hesse-Cassel	Blood sample	782	.	C	T	.	.	.	.	T	T	.	.	.	G	.	.	G	.	.	C

Establishing family



"Relative 1"

"Relative 2"

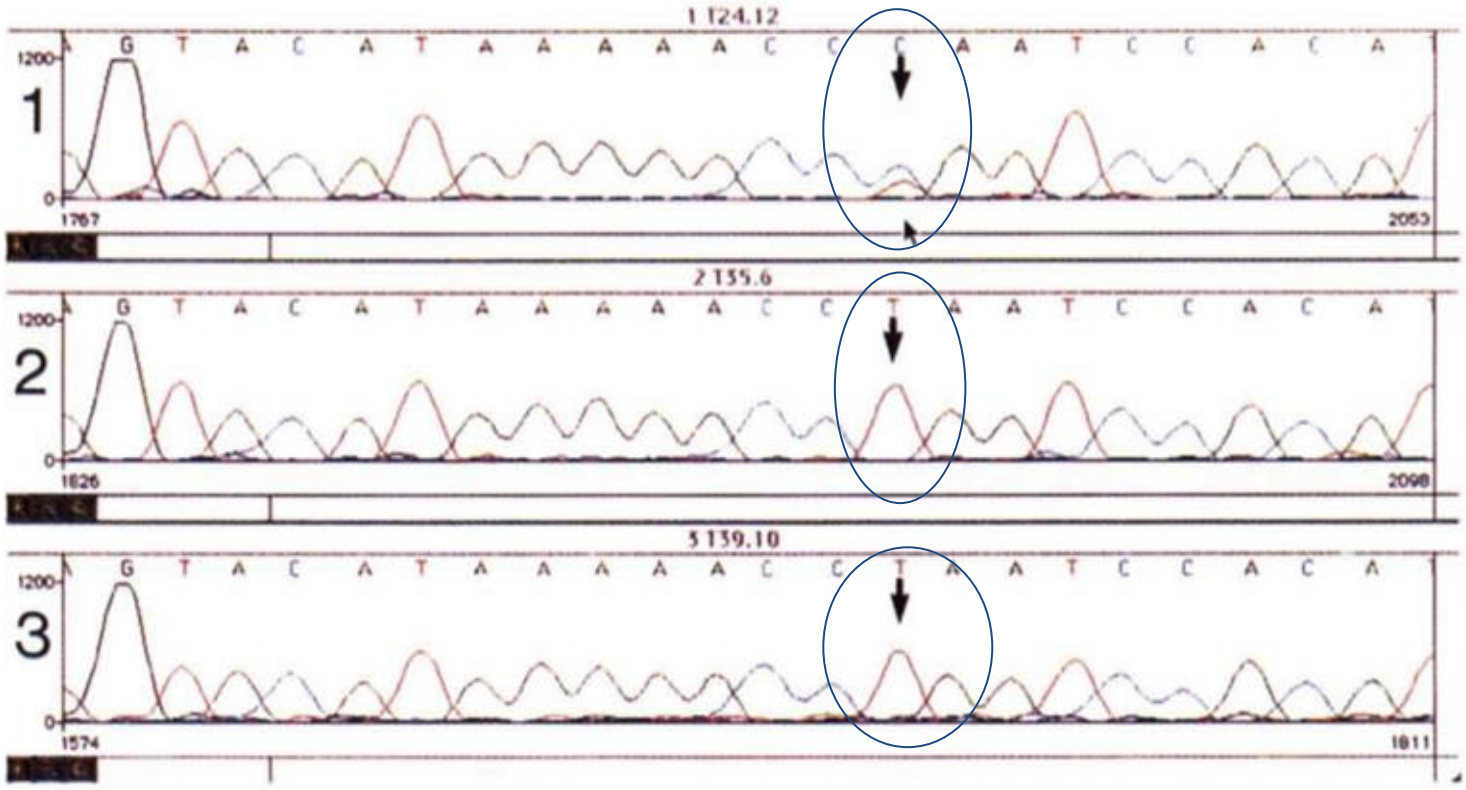
- Two relatives living of unbroken maternal descent
- Same sequence, **except** single nucleotide at position 16169
- Heteroplasmy at this point
  - More than one sequence of DNA present

Establishing family

Tsar Nicholas II

Relative 1

Relative 2



Statistical Analysis

- Two possibilities
  - This **is** the Romanov family
  - This **is not** the Romanov family
- Bayesian Inference
- Likelihood ratios
  - 70 times more likely Tsar had mutation than skeletons unrelated but only differ by single bp

Mystery solved

(Foma.ru)

- Body of younger brother of Tsar Nicholas II exhumed and tested
- Had the same heteroplasmy
- 1.3×10^8 times more likely Romanovs than another family

Anna Anderson

- Claimed to be Alexandria
- Institutionalized after pulled from Berlin river
- Died 1984
- Hair and tissue DNA tested
  - Unrelated to Romanovs
  - Polish Franziska Schanzkowska

Mystery solved (?)

(Research gate)

- 2007: Bone fragments found 70m from original grave site
- Most likely Alexei and missing sister
- Similar analysis, plus Y-DNA and nuclear DNA confirmed relationship
- Re-analyzed old samples with 15 STR loci

Thanks for listening!


(The New York Times)

Questions?