UNIVERSITY of VIRGINIA

7 October 2022
Charlottesville, VA

2022 Chemistry Department Open House & Graham Lecture

Understanding Forensic DNA:

Background, Capabilities, and Limitations

John M. Butler, Ph.D.

NIST Fellow & Special Assistant to the Director for Forensic Science

National Institute of Standards and Technology

NIST NATIONAL INSTITUTE OF STANDARDS AND TECHNOLOGY
U.S. DEPARTMENT OF COMMERCE

The University of Virginia

The General Faculty of the University of Virginia
have conferred the degree of

Doctor of Philosophy
Chemistry

upon

John Marshall Butler

who has completed the courses prescribed for this degree,
In Testimony Whereof the General Faculty have caused this
Diploma to be issued, verified by the signatures of the
President of the University and the Dean of the School,
and under the corporate seal of the University, attested
by the Registrar, at Charlottesville, Virginia, this the
twenty-second day of August, 1995 and in the two hundred
twentieth year of the Commonwealth.

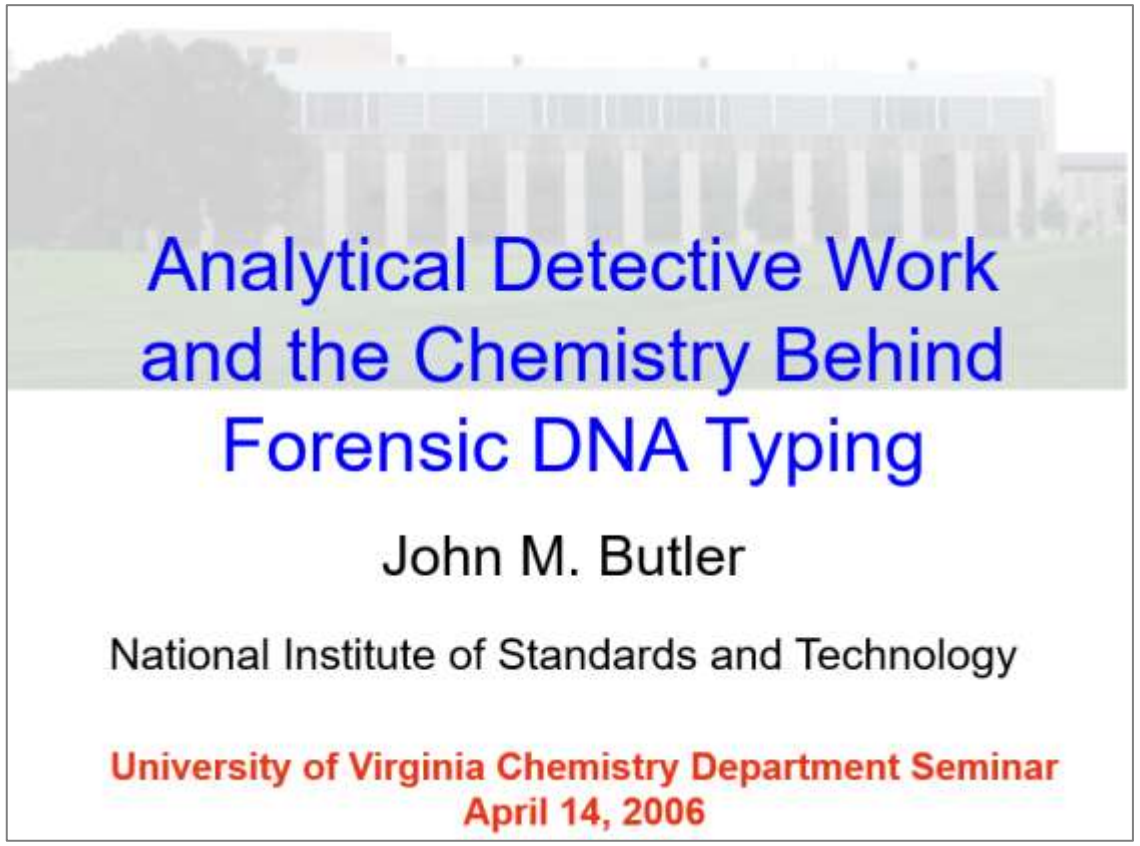
Ph.D. Chemistry

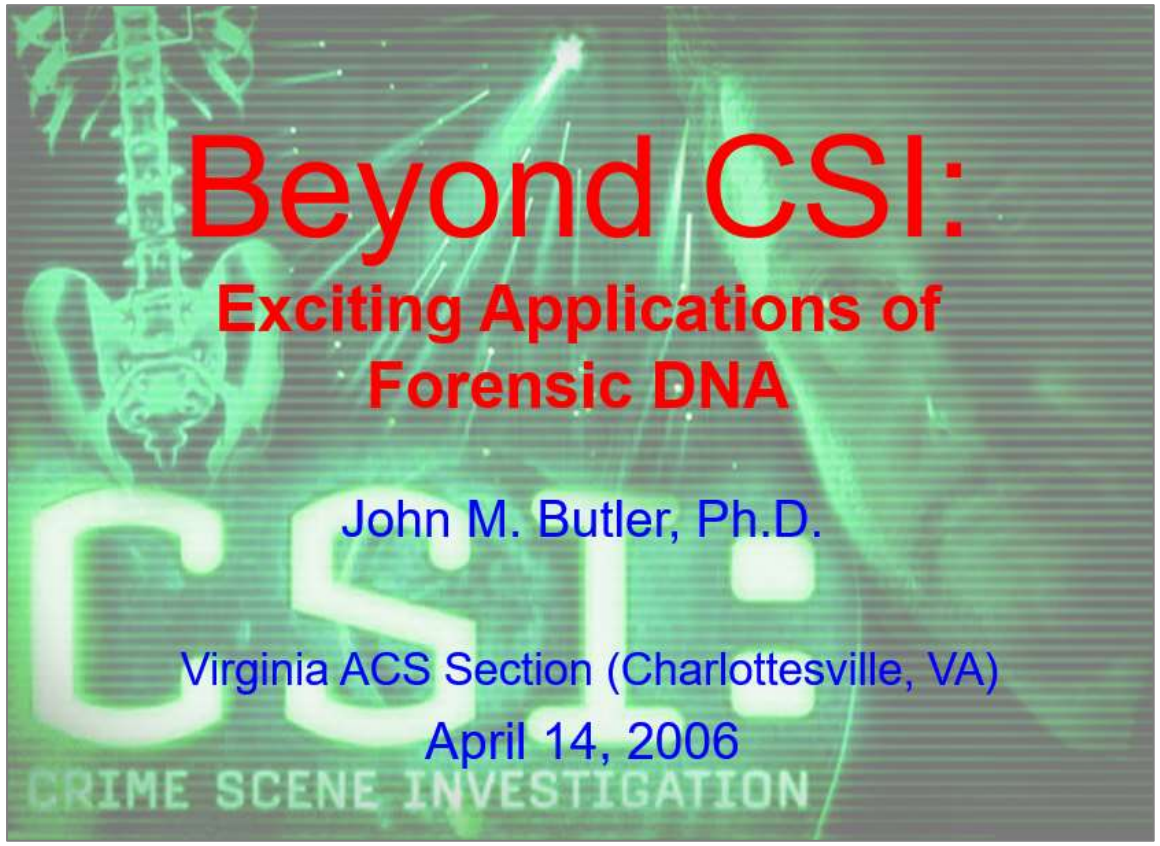
August 1995

Photo courtesy of Professor James Demas

Giving a copy of my 5th book on DNA to my professor, Ralph Allen, on his retirement (**November 2015**)

The Last Time I Spoke at UVA…





A lot has happened in 16.5 years!

My UVA Department of Chemistry Alumni Webpage Needs to Be Updated… ☺

https://chemistry.as.virginia.edu/people/profile/John-Butler

- FACULTY
- AFFILIATED FACULTY
- ADMINISTRATORS & STAFF
- RESEARCH FACULTY & STAFF
- RESEARCH ASSOCIATES
- GRADUATE STUDENTS
- ALUMNI
- EMERITUS AND RETIRED FACULTY
- IN MEMORIAM

JOHN M. BUTLER

NATIONAL INSTITUTE OF STANDARDS AND TECHNOLOGY
FELLOW & GROUP LEADER

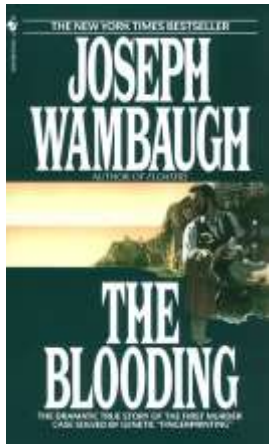
John M. Butler is NIST Fellow and Group Leader of Applied Genetics at the National Institute of Standards and Technology. He is author of the internationally acclaimed textbook Forensic DNA Typing—now in its third edition—as well as more than 100 scientific articles and invited book chapters. His book was also been translated into Chinese (2007) and Japanese (2009). He earned his Ph.D in 1995 from the University of Virginia with Ralph Allen (Analytical Chemistry). His Ph.D. research was conducted in the FBI Laboratory, involved pioneering the techniques now used worldwide in modern forensic DNA testing. Over the past 15 years, Dr. Butler has worked in government and industry. He designed and maintains STRBase (http://www.cstl.nist.gov/biotech/strbase), an information resource for short tandem repeat DNA markers. As a member of the World Trade Center Kinship and Data Analysis Panel, he aided the New York City Office of Chief Medical Examiner in their work to identify the remains of victims of the 9/11 terrorist attacks. He also serves on the

I have a different role at NIST, and my hair is a little grayer now!

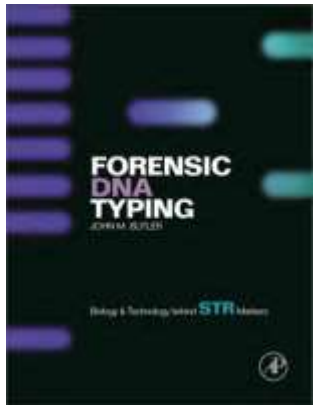
A Brief Overview of My Career

B.S. Chemistry
1992

A friend of my father gave me this book in **1990**, which led to my early interest in forensic DNA



This friend is acknowledged in my first book in **2001**, and given a signed copy shortly after it was published



UNIVERSITY of VIRGINIA

FBI LABORATORY

UVA Grad Student
(Aug 1992- Aug 1995)

Research Conducted at FBI

NIST
National Institute of Standards and Technology

Armed Forces DNA Identification Laboratory

NIST/NRC Postdoc
(Sept 1995- May 1997)

Some Research at AFDIL

GENETRACE

Silicon Valley start-up company doing TOF-MS of DNA

Staff Scientist
(May 1997 – Sept 1999)

NIST
National Institute of Standards and Technology

Human Identity Project Leader (1999-2013)

Research Chemist & Group Leader
(Sept 1999 – Apr 2013)

Special Programs Office (2013-present)

Butler Books on Forensic DNA Typing

For students, practitioners, and lawyers around the world

2015

2012

2010

2005

2001

For the general public

UNDERSTANDING

FORENSIC DNA

SUZANNE BELL & JOHN BUTLER

Cambridge University Press

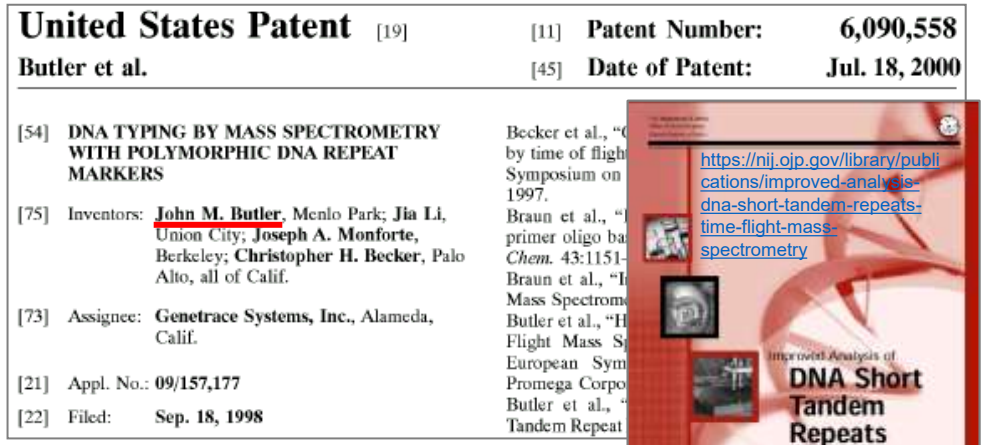
2022

My UVA Graduate Research Proposals

- **DNA MALDI-TOF MS** (Feb 1994 - **failed**)
  - **2 National Institute of Justice grants (>$1M total)**
  - **2 U.S. patents** (GeneTrace Systems; now Sequenom)
  - **12 publications** including 2 articles in the 2006 volume of *The Encyclopedia of Mass Spectrometry*
- **Peptide Nucleic Acids** (April 1994 - **passed**)
  - **3 publications** with PNAs
    - Anal Chem. 1996 Sep 15;68(18):3283-3287
    - Anal Chem. 1997 Dec 1;69(23):4894-4898
    - Nucleic Acids Res. 1999 Dec 15;27(24):4792-4800

This background research conducted in graduate school led directly to successful projects later

…and sometimes you learn more from your failures than from your successes!



United States Patent [19]
Butler et al.

[11] Patent Number: 6,090,558
[45] Date of Patent: Jul. 18, 2000

[54] DNA TYPING BY MASS SPECTROMETRY WITH POLYMORPHIC DNA REPEAT MARKERS

[75] Inventors: John M. Butler, Menlo Park; Jia Li, Union City; Joseph A. Monforte, Berkeley; Christopher H. Becker, Palo Alto, all of Calif.

[73] Assignee: Genetrace Systems, Inc., Alameda, Calif.

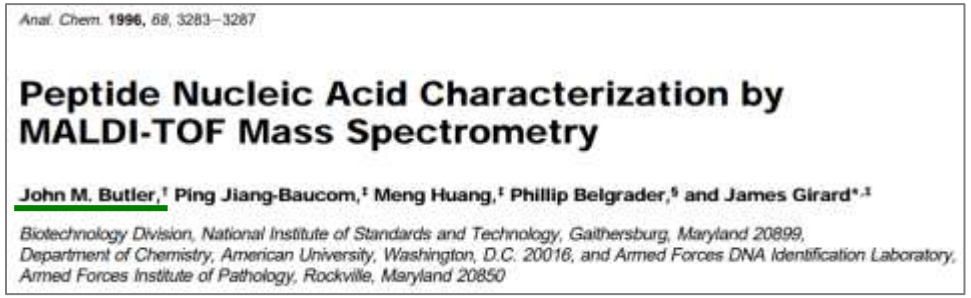
[21] Appl. No.: 09/157,177

[22] Filed: Sep. 18, 1998

https://nij.ojp.gov/library/publications/improved-analysis-dna-short-tandem-repeats-time-flight-mass-spectrometry

Improved Analysis of DNA Short Tandem Repeats With Time-of-Flight Mass Spectrometry

Analytical Chemistry



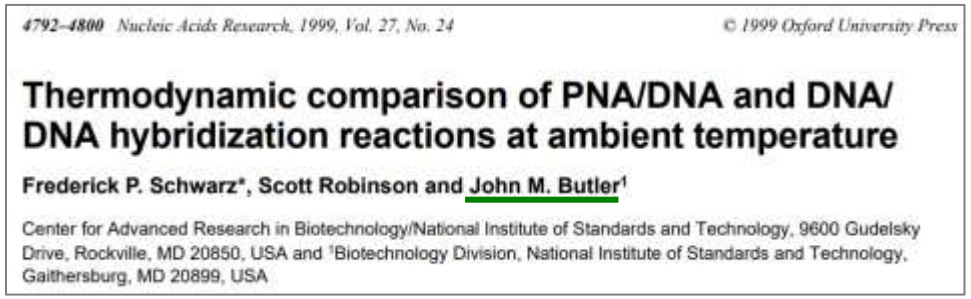
Anal. Chem. 1996, 68, 3283–3287

Peptide Nucleic Acid Characterization by MALDI-TOF Mass Spectrometry

John M. Butler,† Ping Jiang-Baucom,‡ Meng Huang,‡ Phillip Belgrader,§ and James Girard*,‡

Biotechnology Division, National Institute of Standards and Technology, Gaithersburg, Maryland 20899, Department of Chemistry, American University, Washington, D.C. 20016, and Armed Forces DNA Identification Laboratory, Armed Forces Institute of Pathology, Rockville, Maryland 20850

Nucleic Acids Research



4792–4800 Nucleic Acids Research, 1999, Vol. 27, No. 24 © 1999 Oxford University Press

Thermodynamic comparison of PNA/DNA and DNA/DNA hybridization reactions at ambient temperature

Frederick P. Schwarz*, Scott Robinson and John M. Butler†

Center for Advanced Research in Biotechnology/National Institute of Standards and Technology, 9600 Gudelsky Drive, Rockville, MD 20850, USA and †Biotechnology Division, National Institute of Standards and Technology, Gaithersburg, MD 20899, USA

A National Institute of Justice (NIJ) Grant Laid the Foundation for Modern Forensic DNA

A $70,000 NIJ grant awarded to the University of Virginia (UVA) in 1993 literally changed the world of forensic DNA testing — and began my journey into the field of forensic science.

Ralph Allen at UVA and **Bruce McCord** at the FBI Academy's Forensic Science Research and Training Center in Quantico, Virginia, **were looking to apply a new analytical separation technique called capillary electrophoresis (CE) to speed up and automate DNA testing of short tandem repeats (STRs)**. The NIJ grant allowed the two analytical chemists to bring a graduate student on board to work on the new forensic method.

NIJ FUNDING: SUPPORTING EMERGING SCIENTISTS, BUILDING OUR FUTURE

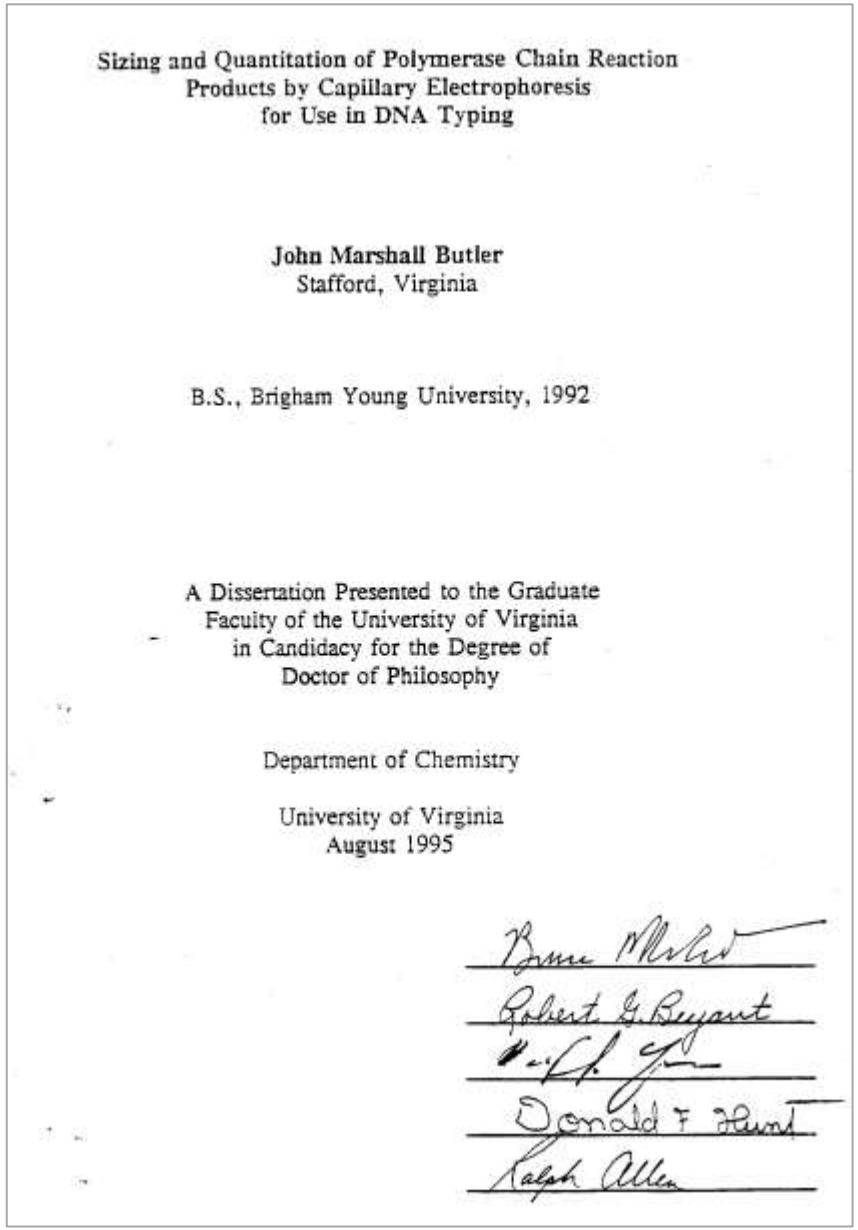
BY **JOHN M. BUTLER**

One influential scientist reflects on the pivotal role that NIJ funding has played during his prolific career in forensic science.

Butler, John M., "NIJ Funding: Supporting Emerging Scientists, Building Our Future," NIJ Journal 276 (2015): 26-31, available at http://www.nij.gov/journals/276/Pages/butler.aspx or https://www.ncjrs.gov/pdffiles1/nij/249224.pdf

Ralph Allen at his UVA retirement in November 2015 with **Alice Isenberg** (PhD, 1998) and **John Butler** (PhD, 1995)

My Ph.D. Dissertation Research



Sizing and Quantitation of Polymerase Chain Reaction Products by Capillary Electrophoresis for Use in DNA Typing

John Marshall Butler
Stafford, Virginia

B.S., Brigham Young University, 1992

A Dissertation Presented to the Graduate Faculty of the University of Virginia in Candidacy for the Degree of Doctor of Philosophy

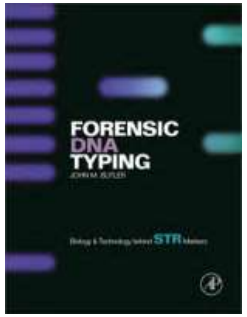
Department of Chemistry

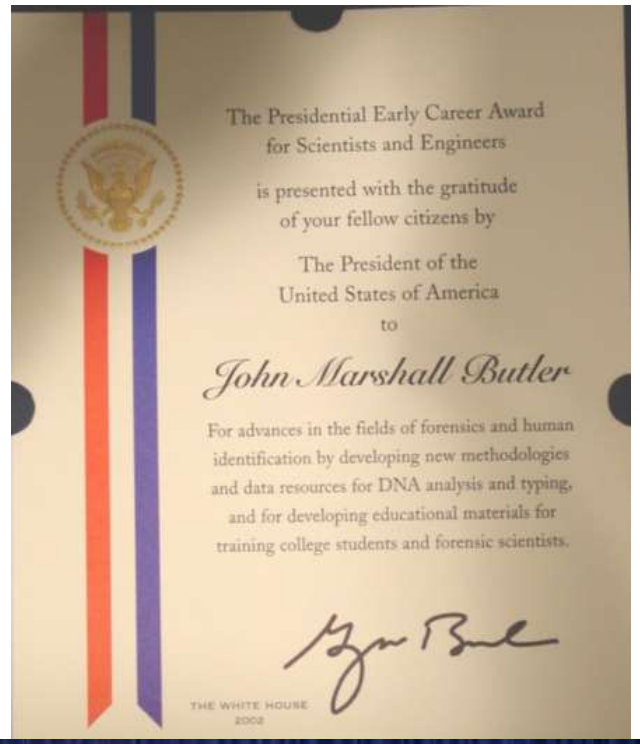
University of Virginia
August 1995

https://strbase.nist.gov/pub_pres/JohnButler-PhD-Dissertation-1995.pdf

- **Pioneered modern forensic DNA testing** through development of capillary electrophoresis separations and detection methods for PCR products
- Provided the initial framework for my first book *Forensic DNA Typing* and the NIST STRBase website (launched in 1997)
- Led to a Presidential Award in July 2002

https://strbase.nist.gov/





In the White House with Arden Bement (former director of NIST and NSF)

Students, what work will you put in to achieve your dream?

Anal. Chem. **1995,** *67,* 273R–294R

Review of the field published in 1995 while I finishing graduate school…

Forensic Science

T. A. Brettell*

Forensic Science Bureau, New Jersey State Police, Box 7068, West Trenton, New Jersey 08625

R. Saferstein

Box 1334, Mount Laurel, New Jersey 08054

Anal. Chem. **2005,** *77,* 3839–3860

10-years later, I was a co-author!

Forensic Science

T. A. Brettell*

Office of Forensic Sciences, New Jersey State Police, New Jersey Forensic Science and Technology Complex, 1200 Negron Road, Horizon Center, Hamilton, New Jersey 08691

J. M. Butler

National Institute of Standards and Technology, Gaithersburg, Maryland 20899-8311

R. Saferstein

Box 1334, Mount Laurel, New Jersey 08054

Every two years (in the June 15th issue until 2011) **the journal *Analytical Chemistry* published an application review on forensic science**

Describes 250 articles covering forensic DNA analysis during 2003-2004

Review Contents

One Measure of Impact in a Scientific Field Relates to Citation of Your Work by Others

8 million scientists
(published between 1960 and 2019)

SCOPUS database (Jan 1996 to Dec 2019)

22 categories
176 subcategories

10,158
in subcategory
Forensic Science
& Legal Medicine

Top 2% = 215

Top Ten

PLOS BIOLOGY

2019

COMMUNITY PAGE

A standardized citation metrics author database annotated for scientific field

John P. A. Ioannidis[1]*, Jeroen Baas[2], Richard Klavans[3], Kevin W. Boyack[4]

1 Departments of Medicine, Health Research and Policy, Biomedical Data Science, and Statistics and Meta-Research Innovation Center at Stanford (METRICS), Stanford University, Stanford, California, United States of America, 2 Research Intelligence, Elsevier B.V., Amsterdam, the Netherlands, 3 SciTech Strategies, Inc., Wayne, Pennsylvania, United States of America, 4 SciTech Strategies, Inc., Albuquerque, New Mexico, United States of America

* jioannid@stanford.edu

PLOS BIOLOGY

2020

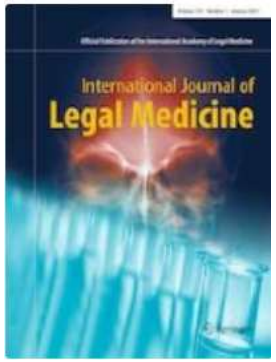
FORMAL COMMENT

Updated science-wide author databases of standardized citation indicators

John P. A. Ioannidis[1,2,3,4]*, Kevin W. Boyack[5], Jeroen Baas[6]

1 Department of Medicine, Stanford University, Stanford, California, United States of America, 2 Department of Epidemiology and Population Health, Stanford University, Stanford, California, United States of America, 3 Department of Biomedical Data Science, Stanford University, Stanford, California, United States of America, 4 Meta-Research Innovation Center at Stanford (METRICS), Stanford University, Stanford, California, United States of America, 5 SciTech Strategies, Inc., Albuquerque, New Mexico, United States of America, 6 Research Intelligence, Elsevier B.V., Amsterdam, the Netherlands

* jioannid@stanford.edu



2021

International Journal of Legal Medicine (2021) 135:701–707
https://doi.org/10.1007/s00414-020-02491-x

ORIGINAL ARTICLE

Scientometric evaluation of highly cited scientists in the field of forensic science and legal medicine

Alan Wayne Jones[1]

Received: 10 November 2020 / Accepted: 15 December 2020 / Published online: 2 January 2021

Jones, A.W. (2021) Scientometric evaluation of highly cited scientists in the field of forensic science and legal medicine. *International Journal of Legal Medicine* 135: 701-707. [published online January 2, 2021]

Top Ten Most Highly Cited Scientists in Forensic Science and Legal Medicine

Table 3 The top ten most highly cited scientists in forensic science and legal medicine among the most highly cited scientists in all scientific disciplines derived from the PLoS Biology article reference [13]

	Scientist	Institute/university	Country	Paper count	Publication years	Rank[1]	Composite score[2] (with self-cites)
	Kintz, P.	University of Strasbourg	France	500	1988–2020	10,321	4.14 (4.20)
DNA	Gill, P.	University of Oslo	Norway	188	1989–2020	16,824	4.01 (4.07)
DNA	Kayser, M.	Erasmus MC, Netherlands	Netherlands	283	1995–2020	19,499	3.97 (4.06)
	Drummer, OH.	Monash University	Australia	285	1976–2020	19,623	3.97 (3.99)
	Jones, AW.	University of Linköping	Sweden	294	1974–2019	20,065	3.96 (4.04)
	Byard, RW.	University of Adelaide	Australia	887	1985–2020	20,467	3.95 (4.19)
DNA	Butler, JM.	National Institute of Standards and Technology	USA	144	1994–2020	22,795	3.92 (3.97)
DNA	Budowle, B.	University of North Texas Health Sciences Center	USA	566	1981–2020	24,019	3.91 (3.98)
DNA	Brinkmann, B.	Institute of Forensic Genetics	Germany	455	1969–2015	27,591	3.87 (3.91)
	Madea, B.	University of Bonn	Germany	730	1984–2020	27,949	3.86 (3.95)

NIST is first from the United States and the only government lab

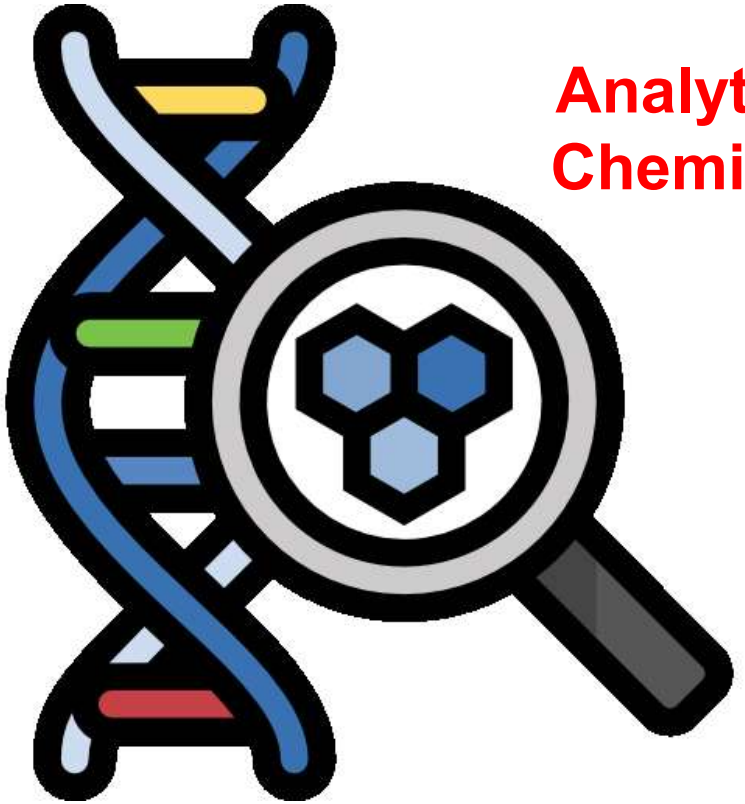
[1] Rank among the top 100,000 most highly cited scientists in all disciplines

[2] Composite score was derived from six citation metrics: (i) total citations, (ii) H-index, (iii) H-index adjusted for co-authorship, (iv) citations to single-author papers, (v) citations to single- or first-author papers, (vi) citations to single-, first-, or last-author papers

Based on articles published from 1960 to 2019 for 8 million scientists (those with ≥5 publications)

My Early Scientific Career Benefited from Straddling Two Disciplines

Analytical Chemistry



Molecular Biology

My first two presentations were to very different audiences:

1. Talk at Fourth Annual **Frederick Conference on Capillary Electrophoresis** (Frederick, MD), October 20, 1993, "Quantitation of PCR Amplified Mitochondrial DNA by Capillary Electrophoresis (CE)"

2. Talk at **American Academy of Forensic Sciences** (San Antonio, TX), February 19, 1994, "Quantitation of PCR Amplified Mitochondrial DNA by Capillary Electrophoresis (CE)"

- **Being comfortable with and operating in multiple domains has made me a more well-rounded scientist and a better communicator**

https://favpng.com/png_view/cell-clipart-dna-molecule-forensic-biology-bloodstain-pattern-analysis-chemistry-png/PAC2R05M


In the home of one of the first forensic analytical chemists
Photo credit: Scott Musgrave
February 2015
221 B Baker Street, London
Looking for evidence of Sherlock Holmes' DNA at his apartment in London

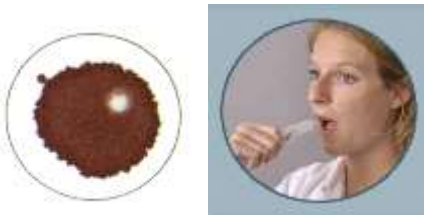
"Seeing" Forensic DNA: Steps in Testing

SAMPLE

Gathering the Data — Understanding Results Obtained

Collection/Storage/ Characterization → Extraction/ Quantitation → Amplification/ Marker Sets → Separation/ Detection → Data → Compare Q→K & Stats → Report

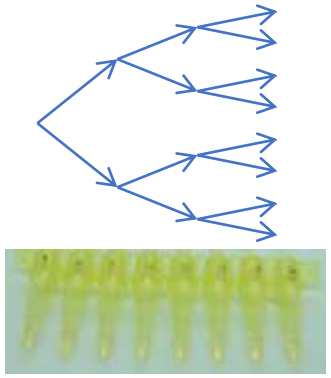
Measurement | **Interpretation** | **Report Issued**



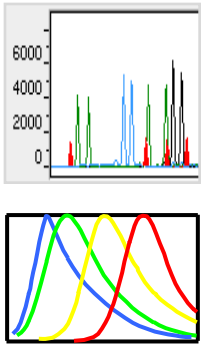
Blood Stain Buccal swab

Sample Collection & Storage

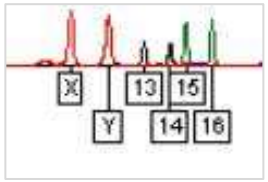
DNA Extraction & Quantitation



Multiplex **PCR** Amplification of STR Markers



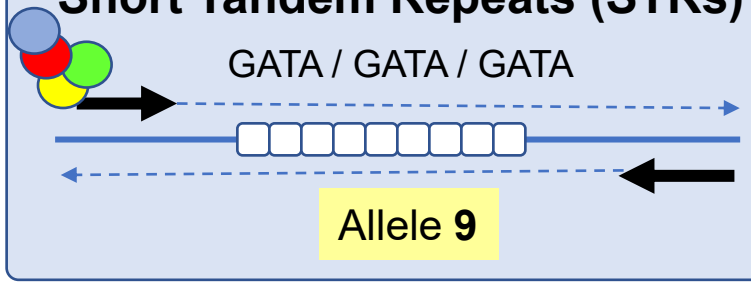
CE with **LIF** Detection



Male: 13,14-15,16-…

Data Interpretation, Statistics & Reporting

Forensic DNA Markers Tested:
Short Tandem Repeats (STRs)



GeneAmp 9700 Thermal Cycler

ABI 3500 Genetic Analyzer
capillary electrophoresis

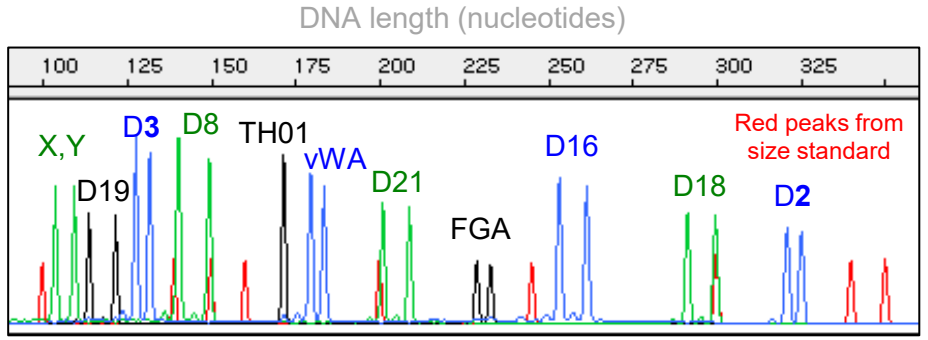
GeneMapper*ID-X* **GeneMarker HID**
software

Probabilistic Genotyping Software

STRmix™
TrueAllele®

Chemistry Involved in Forensic DNA Typing

- **Biochemistry** – DNA extraction; enzymatic amplification (polymerase chain reaction, PCR)
- **Organic chemistry** – fluorescent dye synthesis and oligonucleotide (PCR primer) synthesis
- **Inorganic chemistry** – buffer salts; enzyme cofactors (Mg)
- **Physical chemistry** – PCR primer annealing due to kinetics and thermodynamics of multiplex PCR reaction
- **Analytical chemistry** – size-based electrophoretic separations with capillary electrophoresis and sieving polymers; fluorescent dye spectral emission matrix deconvolution

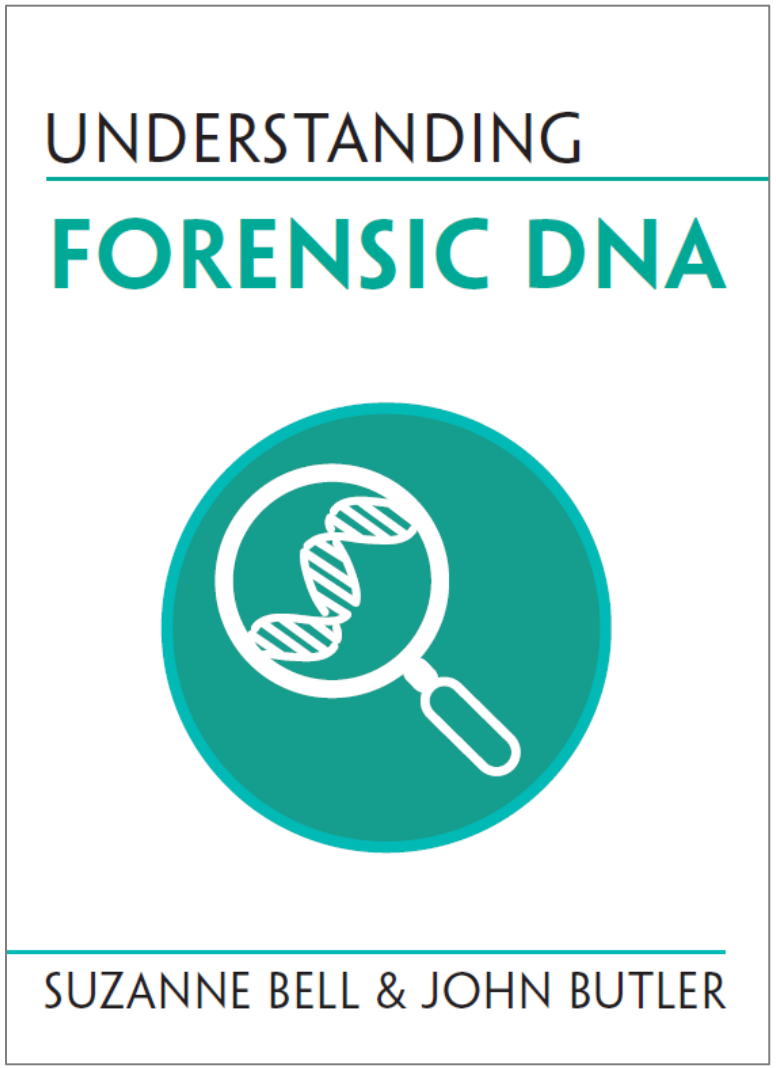


A DNA profile involving 10 **short tandem repeat (STR)** markers labeled with different colored fluorescent dyes during the **polymerase chain reaction (PCR)** amplification process from a sample containing approximately 160 cells (1 ng of genomic DNA)

DNA profile from STR loci

X,Y→ male	**X,Y** Amelogenin
16,17	D**3**S1358
17,18	vWA (chr **12**)
10,12	D**16**S539
22,23	D**2**S1338
12,14	D**8**S1179
28,30	D**21**S11
14,16	D**18**S51
12,14	D**19**S433
6,6	TH01 (chr **11**)
21,22	FGA (chr **4**)

Understanding Forensic DNA



https://www.amazon.com/Understanding-Forensic-DNA-Life/dp/100904401X/

Cambridge University Press (2022)

Book Chapters

1. Biological Identification
2. Before DNA
3. First-Generation Forensic DNA
4. STR Methods and Loci
5. DNA Analysis and Interpretation: Single-Source Samples and Simple Mixtures
6. The Curse of Sensitivity
7. From Mothers and Fathers
8. Emerging Technologies
9. Emerging Issues

Case Stories Shared in *Understanding Forensic DNA*

	Story	Chapter	Principle Emphasized or Concept Explained
	George Rodriguez (ABO typing)	2	Exclusion requires differentiating characteristics
	First immigration case with DNA	3	Family members can be connected (familial DNA testing)
1	First criminal case: Colin Pitchfork	3	First DNA dragnet (*The Blooding*) and investigation finds a killer
	Richard Buckland	3	First exclusion of an innocent man in police custody
	Monica Lewinsky's blue dress	3	DNA testing can even impact a sitting U.S. President
	O.J. Simpson	3	Sample collection and handling are crucial
	Phantom of Heilbronn	4	DNA-free products are needed to collect, store, and analyze samples
2	Lukis Anderson	6	Potential indirect transfer matter when using sensitive DNA testing
	Amanda Knox	6	Problems with improper collection techniques and touch DNA
3	Romanovs & Anna Anderson	7	Using mitochondrial DNA to identify historical remains and test claims
4	Hemings-Jefferson relationship?	7	Using Y-chromosome DNA to assess historical parentage claims
	King Richard III	8	Assessing recovered remains via ancestry and phenotyping DNA
	Snowball the cat	8	Using cat DNA to associate a crime with the cat's owner
	Grim Sleeper	9	Familial searching to catch a killer with association to his son's DNA
	Golden State Killer	9	Using investigative genetic genealogy to locate a serial killer

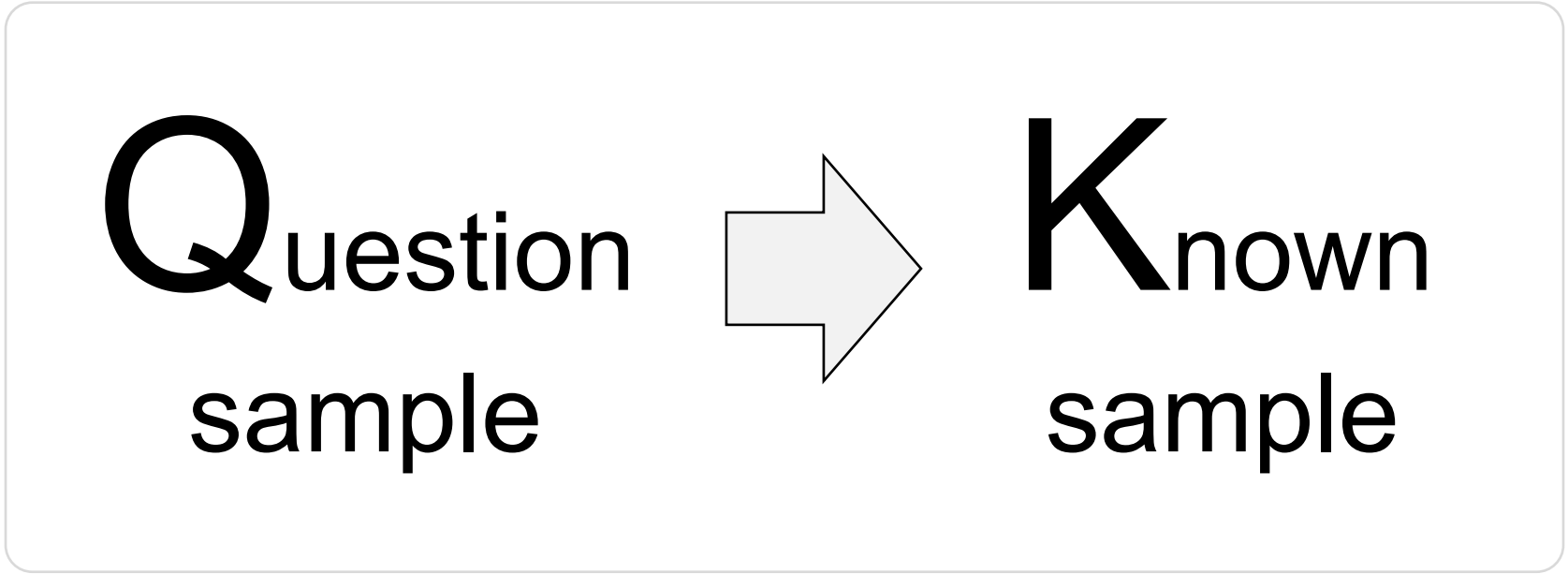
Areas of Human Identification with DNA

- Forensic cases -- **matching suspect with evidence**
- Paternity testing -- **identifying father**
- Missing persons investigations
- Military DNA “dog tag”
- Convicted offender and arrestee DNA databases
- Mass disasters -- **putting pieces back together**
- Historical investigations

Involves generation of DNA profiles usually with the same core STR (short tandem repeat) markers and then MATCHING TO REFERENCE SAMPLE

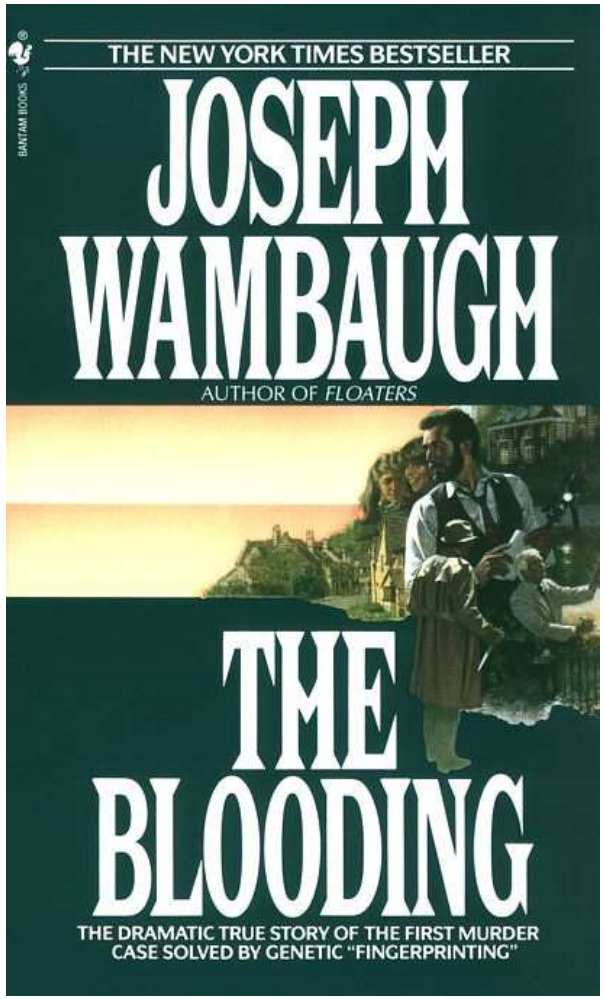
Q → K Comparison

Used in Forensic Science



Forensic case	*Evidence from Crime*	*Suspect(s)*
Paternity testing case	*Alleged Father(s)' DNA Profile*	*Child's DNA Profile*

Lessons from the First Case Involving DNA Testing



Describes the first use of DNA (in 1986) to solve a double rape-homicide case in England; about 5,000 men asked to give blood or saliva to compare to crime stains

- Connection of two crimes (1983 and 1986)
- Use of a DNA database to screen for perpetrator (DNA only done on 10% with same blood type as perpetrator)
- Exoneration of an innocent suspect *(Richard Buckland)*
- DNA was an investigative tool – did not solve the case by itself (confession of accomplice led to the killer)

Lynda Mann (1983)

Dawn Ashworth (1986)

Colin Pitchfork

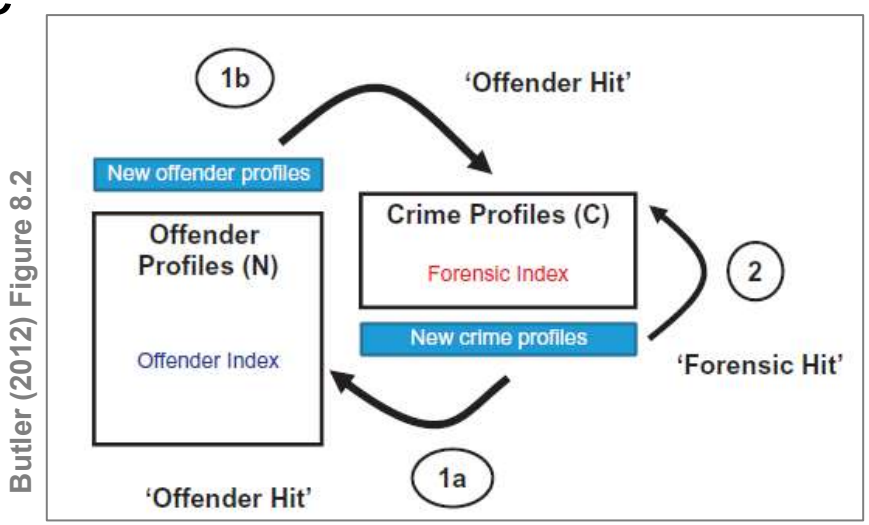
A local baker, Colin Pitchfork, was arrested and his DNA profile matched with the semen from both murders. In 1988 he was sentenced to life for the two murders.

U.S. National DNA Database

National DNA Index System (NDIS)

- Authorized under DNA Identification Act of 1994
- Since 1998, maintained by FBI Laboratory with input from **~200 local (LDIS) and state (SDIS) laboratories**
- Categories of crimes for **inclusion based on state laws**
- Laboratories are audited to the **FBI Quality Assurance Standards** (1998/1999, 2009, 2011, and 2020)
- Uses CODIS (Combined DNA Index System) software
- **Requires 20 STR loci** to provide a common currency
- Statistics as of October 2021*:
  - 14,836,490 offenders
  - 4,513,955 arrestees
  - 1,144,255 forensic profiles
- **Assisted 574,343 investigations**

~35% hit rate
(per ISHI 2021 Q&A)



*https://le.fbi.gov/science-and-lab-resources/biometrics-and-fingerprints/codis/codis-ndis-statistics

What DNA Testing Can and Cannot Do

Can

1. **Answer the question of *who* left the DNA** (best results are with high-quantity, single-source DNA)
2. **Confirm a possible genetic relationship with biological relatives** (e.g., paternity testing)
3. With a different type of testing, **assist investigations through noting distant family relationships** (e.g., investigative genetic genealogy)
4. Due to high sensitivity testing, **generate DNA results that may not be relevant to a case** because of contamination or DNA transfer

Cannot

1. **Answer the questions of *when* (or *how*) the DNA was deposited**
2. Prove that someone **did commit a crime** with the presence of DNA
3. Prove that someone **did not commit a crime** with the absence of DNA

Lukas Anderson (2012): The Impact of DNA Transfer

Photo from Los Gatos-Monte Sereno Police Department

In November 2012, a group of men broke into a Silicon Valley mansion; the 66-year-old owner died after being tied up

Photo from San Mateo County Crime Laboratory

Lukis Anderson's DNA was found on fingernail clippings from the victim's right hand

Photo by Carlos Chavarria

Lukis Anderson, a homeless alcoholic in nearby San Jose, CA, was then charged for the murder of the victim Raveesh Kumra

Photo by Carlos Chavarria

Erin Lunsford of the Los Gatos-Monte Sereno Police Department was the lead investigator in the murder of Raveesh Kumra

Photo by Carlos Chavarria

Public defender Kelley Kulick discovered that **Anderson had been in the hospital passed out drunk at the time of the murder**

[Roland] van Oorschot, the forensic science researcher whose 1997 paper revolutionized the field, cautions against disbelieving too much in the power of touch DNA to solve crimes. "I think it's made a huge impact in a positive way," he says. "But **no one should ever rely solely on DNA evidence to judge what's going on**."

Three hours after picking up Anderson to take him to the hospital because he was passed out drunk, the same two paramedics responded to the Kumra mansion, where they checked the murder victim's vitals with the same pulse oximeter likely enabling Anderson's DNA to transfer to Kumra's right index fingernail

https://www.thrivespc.com/home-medical-solutions/pulse-oximeter/

pulse oximeter

https://www.pbs.org/wgbh/frontline/article/framed-for-murder-by-his-own-dna/

DNA Transfer Can Potentially Impact Investigations of Firearms Possession

Forensic Science International: Genetics 48 (2020) 102355

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsigen

Research paper

DNA transfer to firearms in alternative realistic handling scenarios

Annica Gosch, Jan Euteneuer, Johanna Preuß-Wössner, Cornelius Courts*

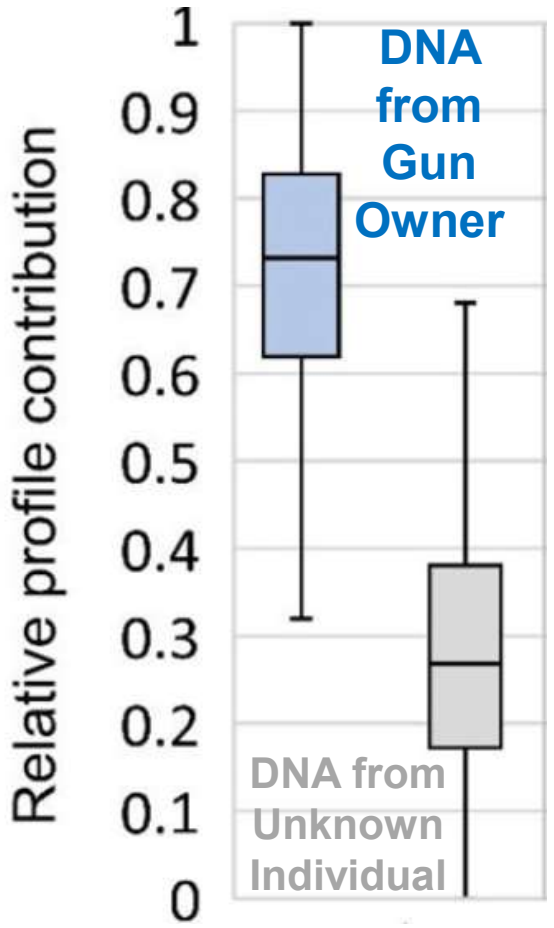
Institute of Forensic Medicine, University Medical Center Schleswig-Holstein, Kiel, Germany

Examined DNA mixtures from skin contact traces of DNA recovered from three surfaces of two types of firearms handled in four realistic, casework-relevant handling scenarios

ABSTRACT

Firearms are the most relevant items of evidence in gun-related crimes, likely bearing various traces facilitating an objective reconstruction of the crime. Trace DNA recovered from firearm surfaces might help to identify individual(s) having handled the firearm and thereby possibly to link the firearm and the corresponding shooter, however, the interpretation of DNA traces on handled items can be challenging and requires a detailed understanding of various factors impacting DNA prevalence, transfer, persistence and recovery. Herein, we aimed at improving our understanding of factors affecting the variability of trace DNA characteristics recovered from firearms handled in gun-related crimes: Skin contact traces were recovered from various outer surfaces of two types of firearms handled in four realistic, casework-relevant handling scenarios and the corresponding trace characteristics (DNA yield, number of contributors, relative profile contribution for known and unknown contributors, LRs) were compared. Trace DNA characteristics differed distinctly between handling conditions, firearm and surface types as well as handling individuals and intraindividual deposits emphasizing the variability and complexity of trace DNA profile composition expected to be recovered from firearms after realistic handling scenarios. The obtained results can provide useful insights for forensic experts evaluating alternative activity level propositions in gun-related crimes.

Only the owner supposedly handled this "clean" gun

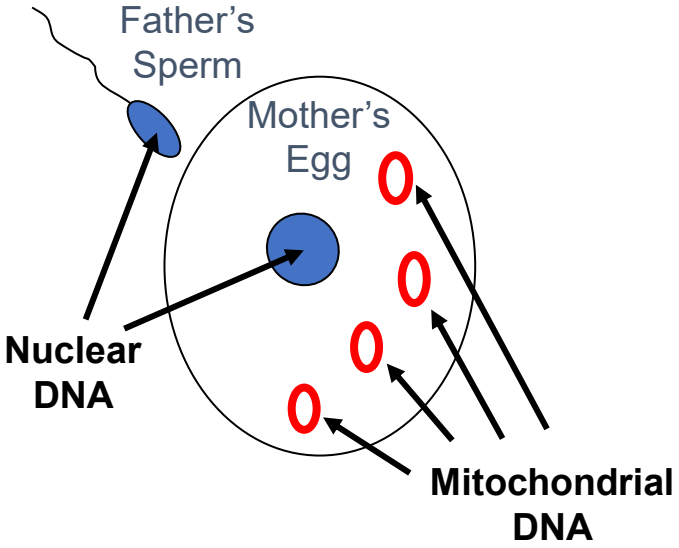


Each test was repeated three times with two different owner/shooter pairs

Different Inheritance Patterns

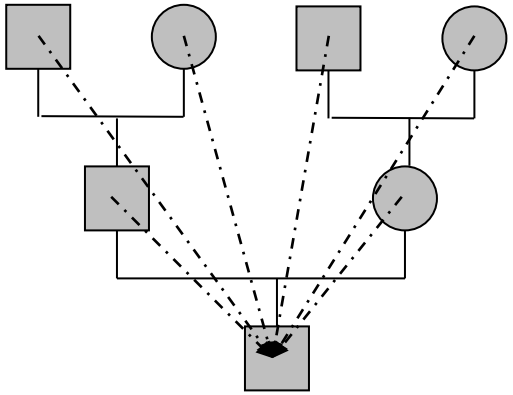
= **males** (X,Y)

= **females** (X,X)



Autosomal Markers

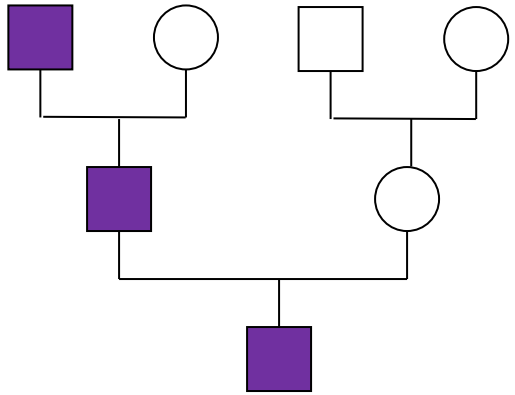
Autosomal STR Loci



22 pairs of autosomes
(passed on in part,
from all ancestors)

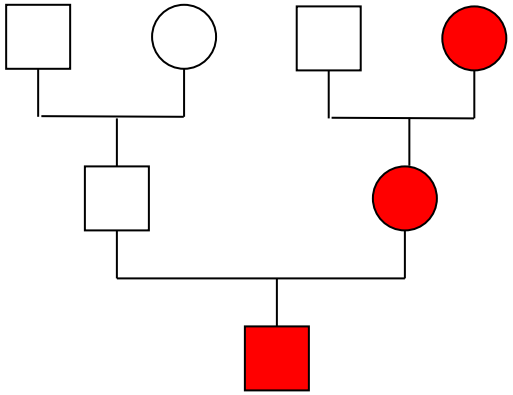
Lineage Markers

17, 23, or 27 Y-STRs



Y-Chromosome
(passed on complete,
but only by sons)

mtDNA control region



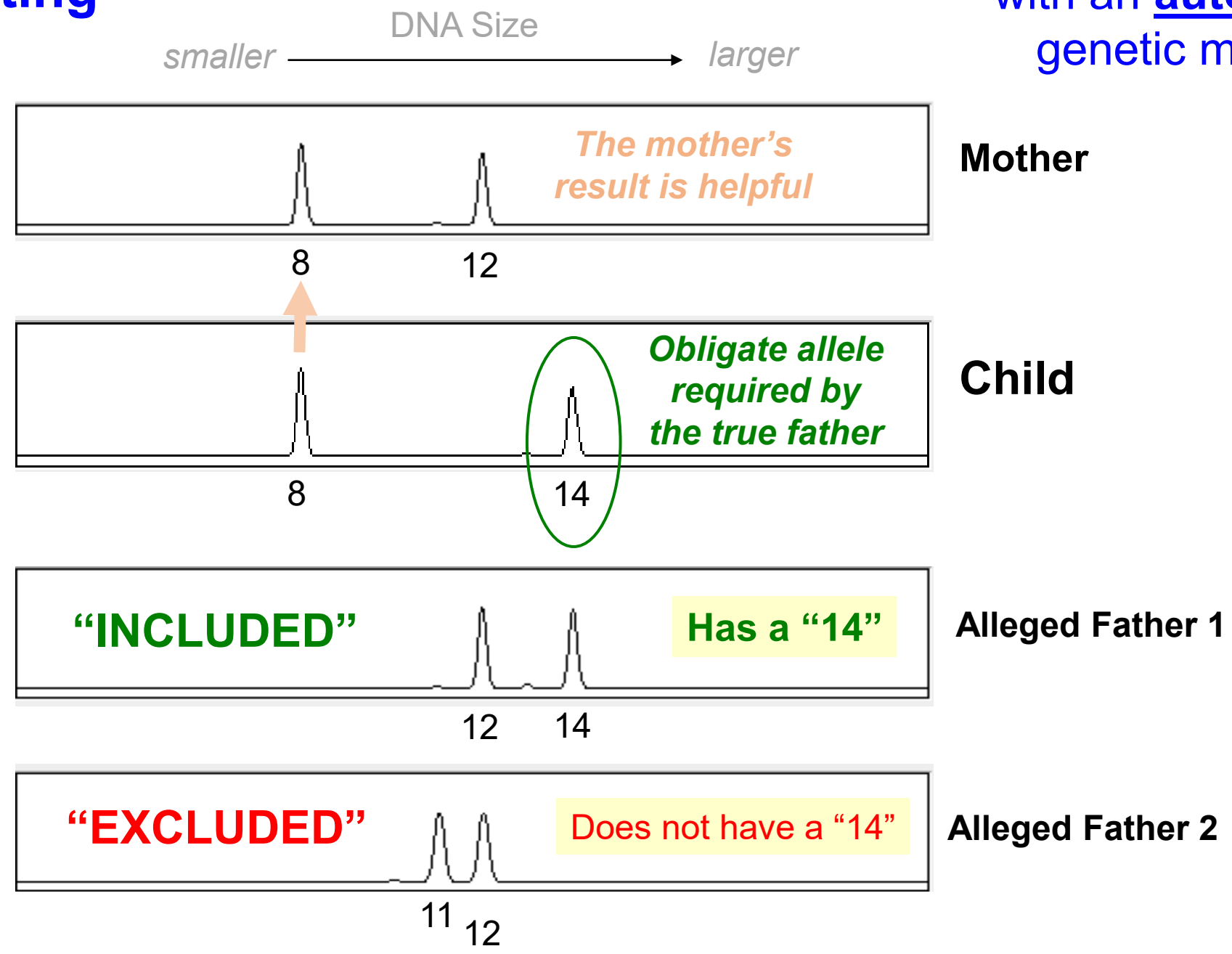
Mitochondrial
(passed on complete,
but only by daughters)

Father contributes: 22 autosomes (1 of each pair), X or **Y**
Mother contributes: 22 autosomes (1 of each pair), X and **mtDNA**

From Figure 15.1 in Butler, J.M. (2015) *Advanced Topics in Forensic DNA Typing: Interpretation* Elsevier Academic Press: San Diego

Paternity Testing Example

with an **autosomal** genetic marker



Complicating Factors in Genetic Inheritance

- **Recombination with autosomal DNA**
  - Reshuffling process at each generation (which leads to genetic variation) complicates the signal observed in the next generation
  - Ambiguity increases with the number of generations apart the individuals are that are being compared
- **Random chance of inheritance with autosomal DNA**
  - Which one of the two parents provides the inherited allele varies with each child
  - Overtime the original signal is lost to that portion of the family tree
- **Mutations**
  - Mutations can change inherited alleles (e.g., 15 → 14 or 15 → 16)
  - The more markers tested, the more likely a mutation will be observed
  - SNPs mutate less frequently than STRs

Lineage markers (Y-STRs and mtDNA) are not impacted by recombination or random inheritance issues and are thus helpful in making "long-distance" associations across multiple generations

The Romanovs – Russia's Last Royal Family

http://upload.wikimedia.org/wikipedia/en/thumb/9/9f/The_Romanovs_-_1913.jpg/330px-The_Romanovs_-_1913.jpg

Photo taken in 1913 five years before they were killed
on July 17, 1918, by the Bolsheviks (the Red Army)

Began working on the burial site in July 1991 and discovered about 1000 bone fragments but only 9 skulls whereas 11 people had been murdered

Gill et al. (1994) *Nature Genet*. 6(2):130-135

Identification of the remains of the Romanov family by DNA analysis

Peter Gill[1], Pavel L. Ivanov[2], Colin Kimpton[1], Romelle Piercy[1], Nicola Benson[1], Gillian Tully[1], Ian Evett[1], Erika Hagelberg[3] & Kevin Sullivan[1]

Follow-up article: Ivanov et al. (1996) Mitochondrial DNA sequence heteroplasmy in the Grand Duke of Russia Georgij Romanov establishes the authenticity of the remains of Tsar Nicholas II. *Nature Genet.* 12(4):417-420

But the remains of son Alexei and one of the daughters (Anastasia or Maria) were not originally recovered

The Romanovs (Last Russian Czar) were originally identified using mitochondrial DNA

Photo courtesy of Mike Coble (Armed Forces DNA Identification Laboratory)

Romanov Remains Laid to Rest on July 17, 1998

(80 years to the day they were killed)

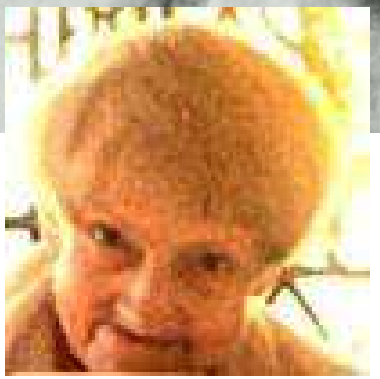
Prince Philip (Queen Elizabeth's husband) was a maternal relative to Tsarina Alexandra

SOURCES: Gill et al. (1994) *Nature Genetics,* 6, 130-135.; Ivanov et al. (1996) *Nature Genetics,* 12, 417-420; Stone, R. (2004) *Science*, 303, 753.

Charlottesville resident Anna Anderson Manahan was likely Franziska Schanzkowska

http://en.wikipedia.org/wiki/Anna_Anderson

http://en.wikipedia.org/wiki/Image:Annaan.JPG



Died in 1984 in Charlottesville

The DNA tests showed that Anderson's DNA did not match the Romanov remains or Prince Philip, Duke of Edinburgh (a relative of the Romanovs), but was **consistent with the mitochondrial DNA profile of Karl Maucher, a great-nephew of Franziska Schanzkowska**

Romanov & Prince Philip
Mitotype
16111T
16357C
263G
315.1C

Anderson & Maucher
Mitotype
16126C
16266T
16294T
16304C

Establishing the identity of Anna Anderson Manahan

Sir — The 70-year controversy surrounding the identity of Anna Anderson Manahan has been remarkable in capturing the imagination of so many people. Supporters of her claim said that she was the Duchess Anastasia, daughter of Tsar Nicholas II and had dramatically escaped execution by a Bolshevik firing squad on July 16th, 1918. Disbelievers denied the claim, but could not establish her true identity.

About the same time, Anna Anderson appeared and claimed to be the Royal Duchess Anastasia.

Following the Forensic Science Service (FSS) work last year[1] to identify the remains of the Tsar and his family, the FSS and the Armed Forces Institute of Pathology (AFIP) were asked to carry out independent analyses on small bowel samples removed from Anna Anderson Manahan during the course of surgery

DNA profiles analysed from bones of the Tsar and Tsarina (Table 1); four out of five different STRs were inconsistent with the hypothesis of parentage. In addition, we carried out sex typing of the tissue using the amelogenin test[7].

The non-coding region of mtDNA was sequenced and compared to a blood sample donated by HRH the Duke of Edinburgh, a direct maternal descendant of the Tsarina. We found

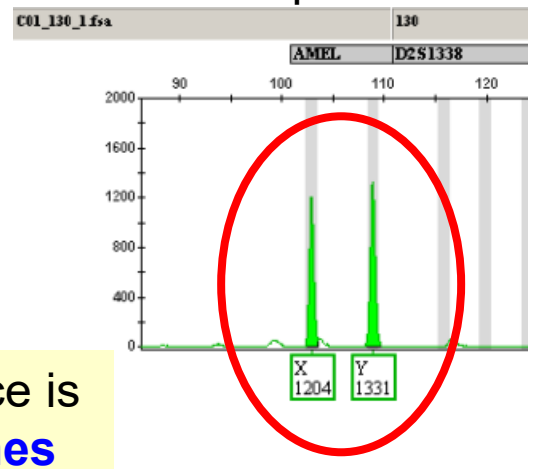
Stoneking et al. (1995) Establishing the identity of Anna Anderson Manahan. *Nature Genet*. 9(1):9-10

Some of the Recovered Remains from Grave Site Discovered in July 2007

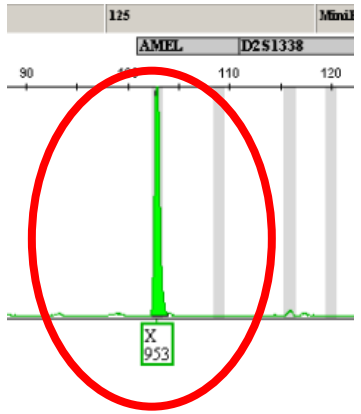
One Sample is Male and the Other Female

The DNA evidence is **5.63 million times** more likely IF samples 146 and 147 are siblings than if these samples were from two unrelated individuals

Sample 146



Sample 147



A few of the charred bone fragments + one nail

Data courtesy of Mike Coble
(Armed Forces DNA Identification Laboratory)

Can These Remains be Children of Tsar Nicholas II and Tsarina Alexandra?

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

[a]Allele designation for all loci except HUMACTBP2 is based on the number of repeat units (determined by sequencing of specific alleles — data not shown). The allele designation for HUMACTBP2 is based on an arbitrary scale identical to that of Kimpton *et al.*[2].

Nature Genetics volume 6 february 1994

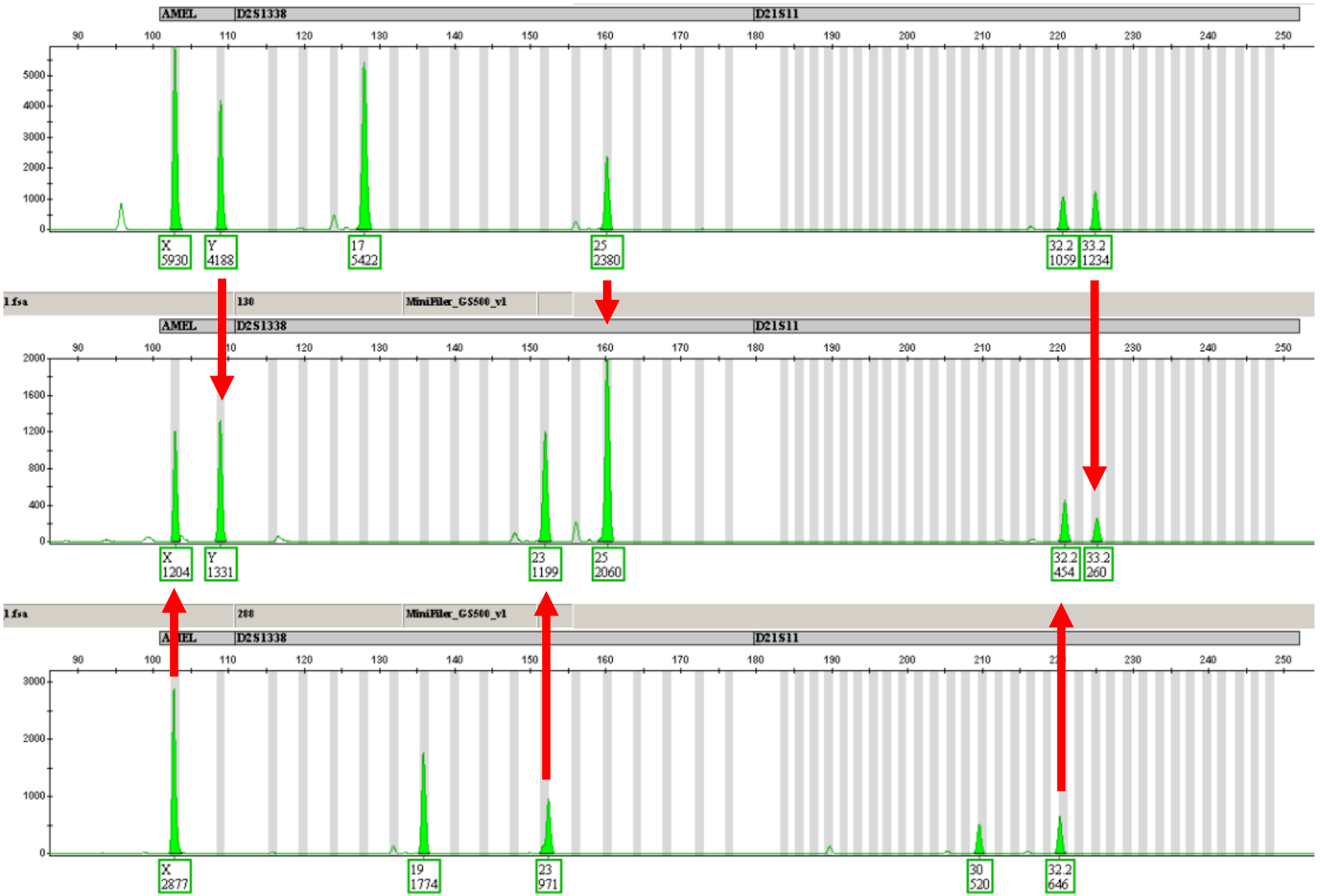
Gill *et al.* (1994)

Slide courtesy of Mike Coble (Armed Forces DNA Identification Laboratory)

Tsar

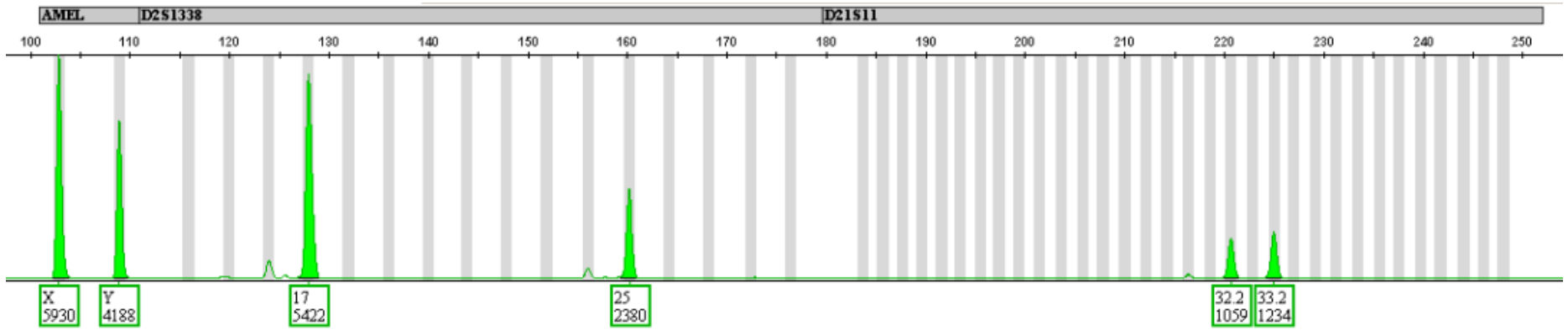
146.1

Tsarina

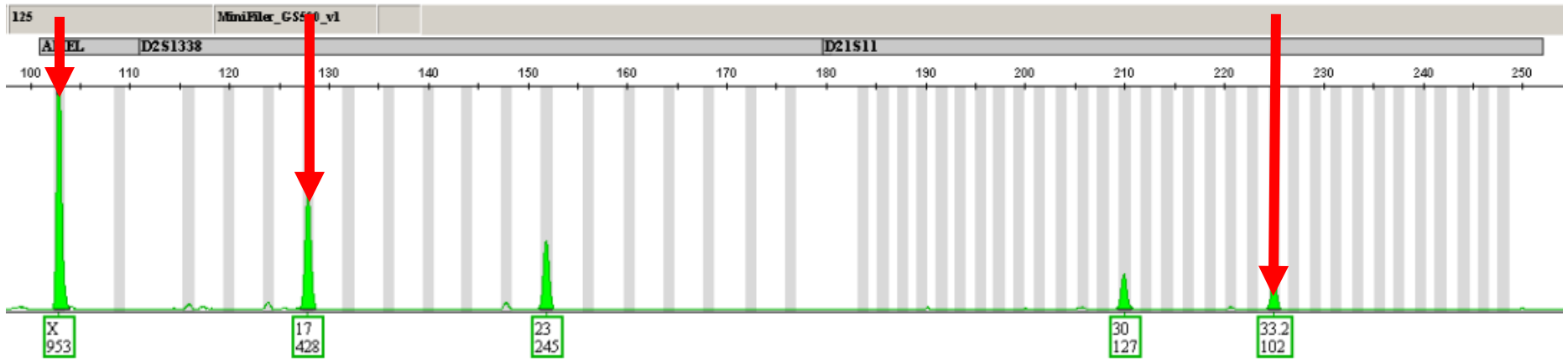


Slide courtesy of Mike Coble (Armed Forces DNA Identification Laboratory)

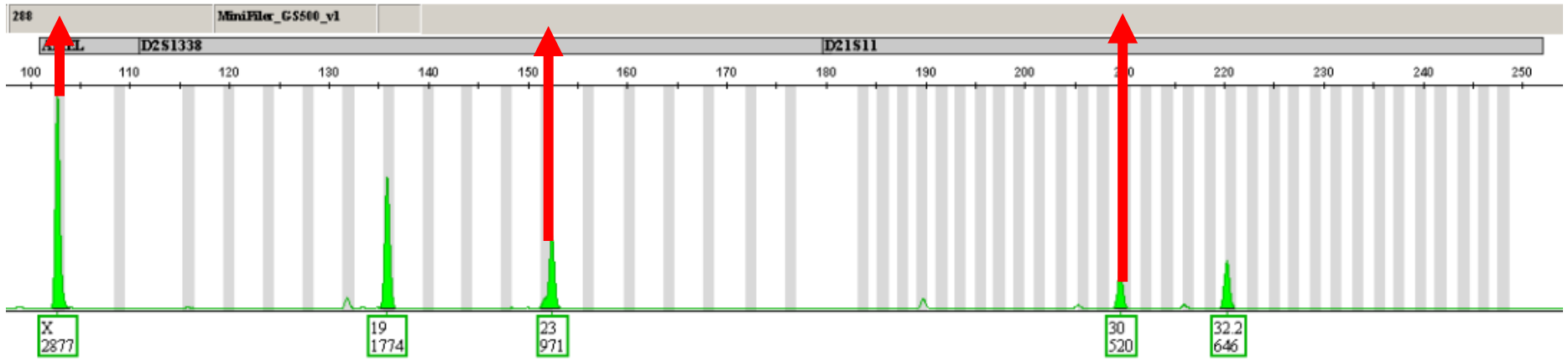
Tsar



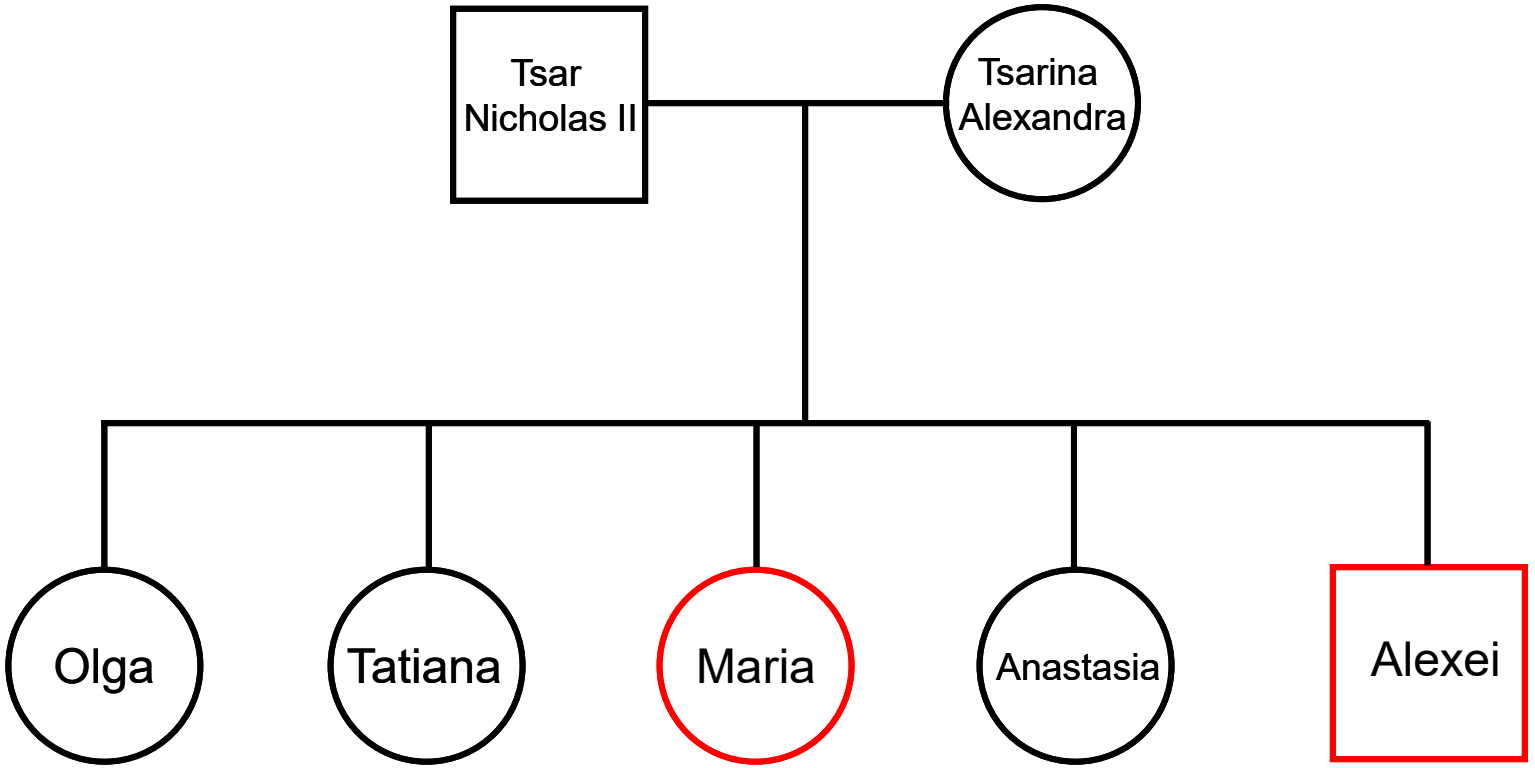
147



Tsarina



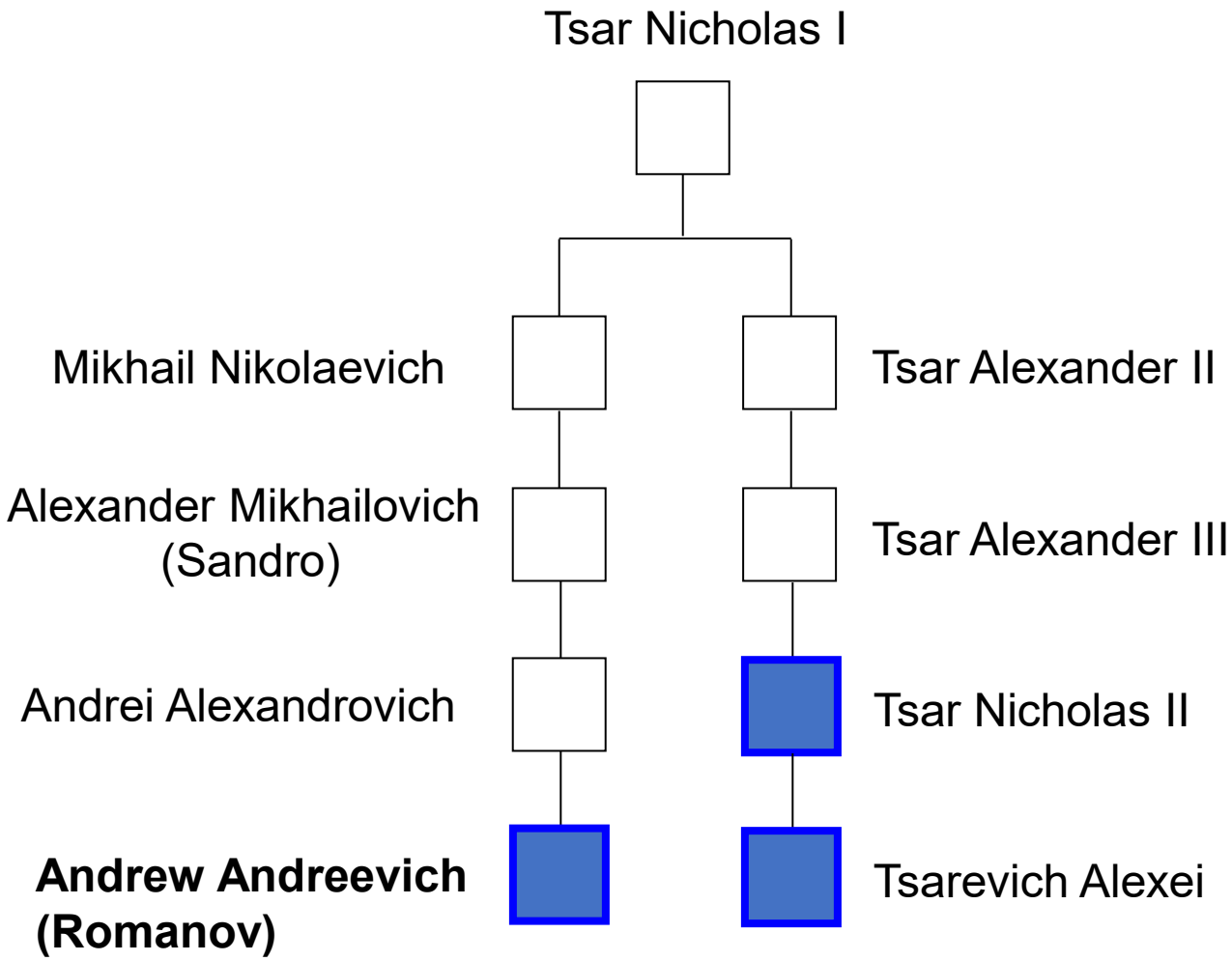
Slide courtesy of Mike Coble (Armed Forces DNA Identification Laboratory)



It is **4.36 trillion times** more likely IF samples 146.1 and 147 belong to this family pedigree than if these samples were from two unrelated individuals selected from the population.

Slide courtesy of Mike Coble (Armed Forces DNA Identification Laboratory)

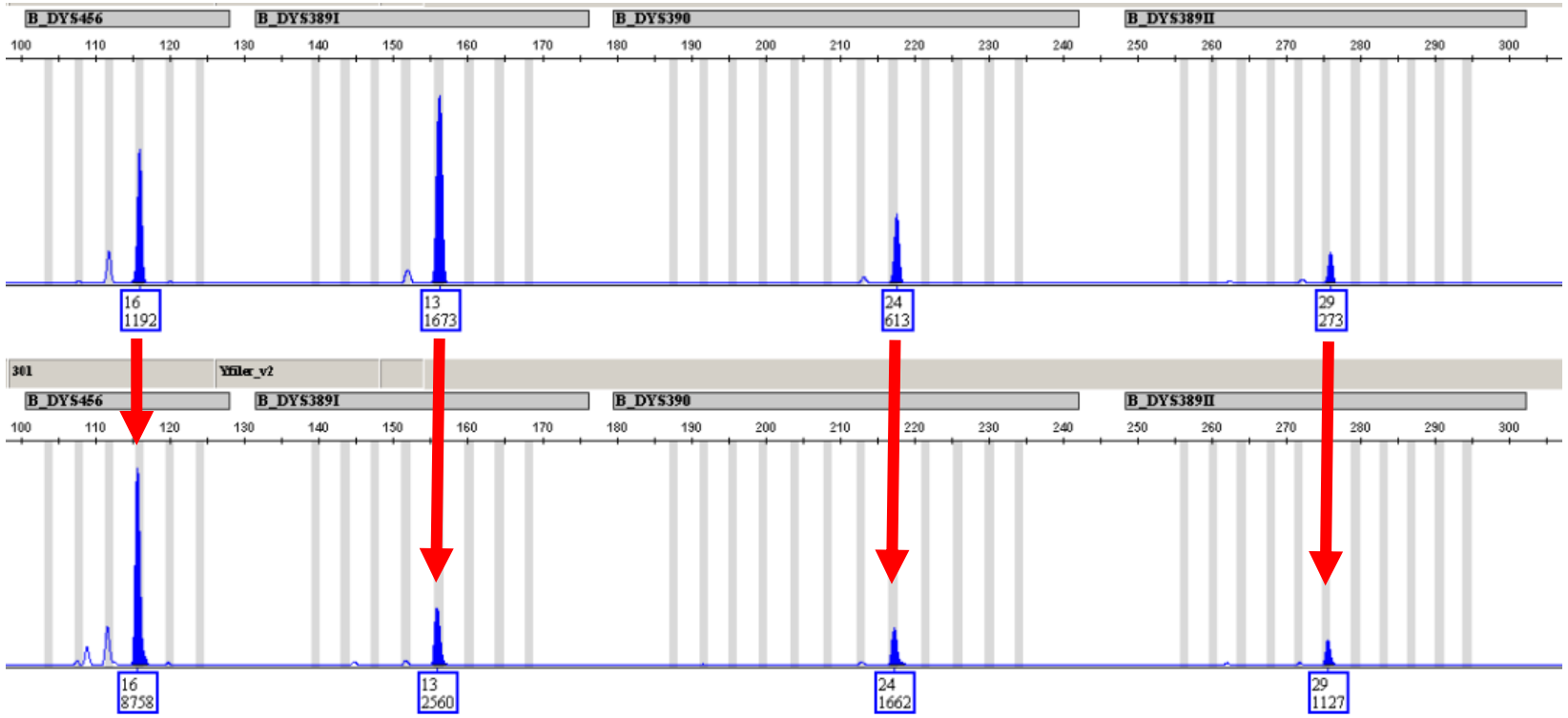
A Living Male Relative Was Found to Confirm Y-Chromosome Testing Results



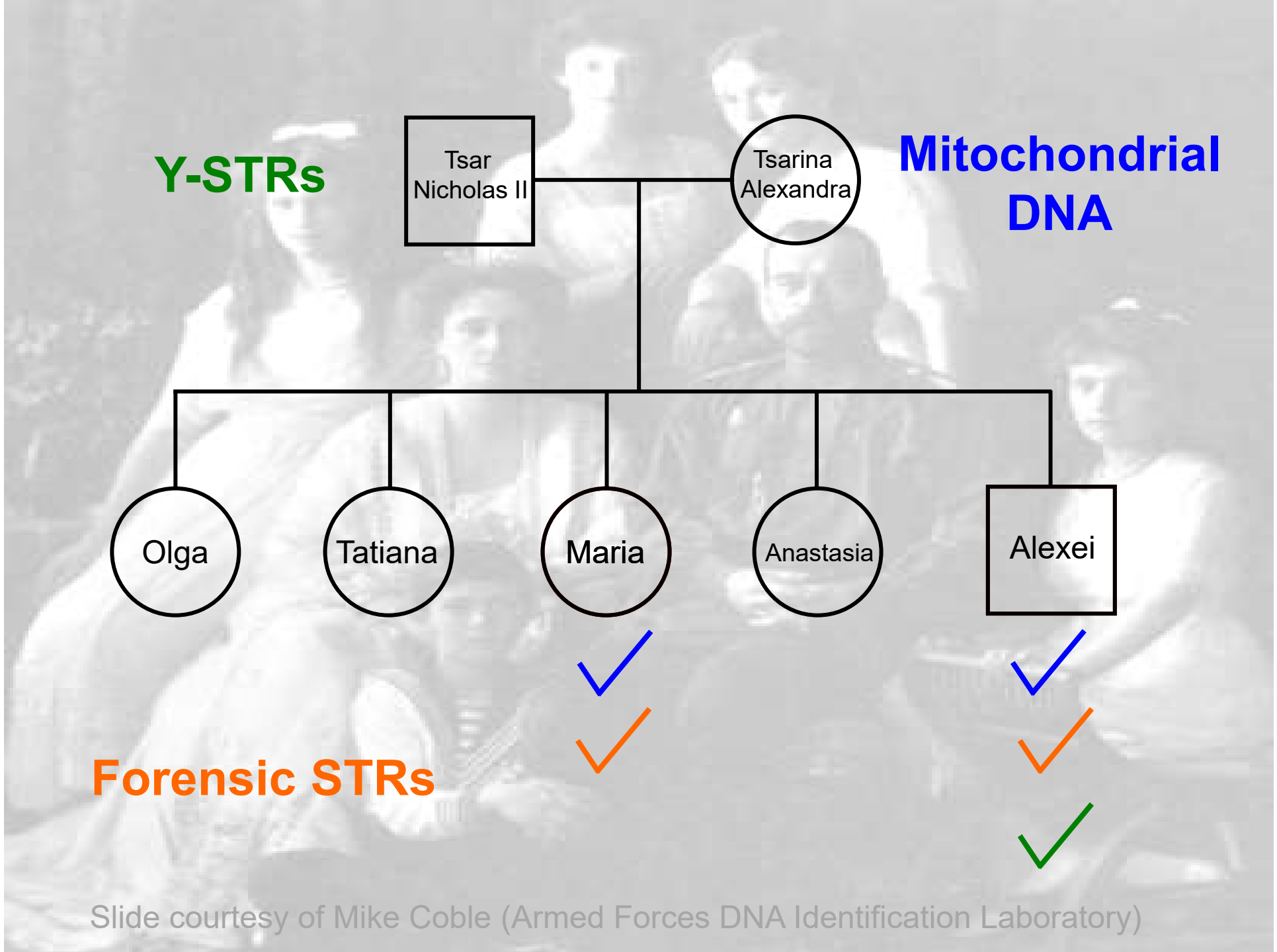
Slide courtesy of Mike Coble (Armed Forces DNA Identification Laboratory)

Alexei

Tsar Nicholas II



Slide courtesy of Mike Coble (Armed Forces DNA Identification Laboratory)



Slide courtesy of Mike Coble (Armed Forces DNA Identification Laboratory)

Article published in March 2009

OPEN ACCESS Freely available online

http://www.plosone.org

PLoS one

Mystery Solved: The Identification of the Two Missing Romanov Children Using DNA Analysis

Michael D. Coble[1☯*], Odile M. Loreille[1☯], Mark J. Wadhams[1], Suni M. Edson[1], Kerry Maynard[1], Carna E. Meyer[1], Harald Niederstätter[2], Cordula Berger[2], Burkhard Berger[2], Anthony B. Falsetti[3], Peter Gill[4,5], Walther Parson[2], Louis N. Finelli[1]

1 Armed Forces DNA Identification Laboratory, Armed Forces Institute of Pathology, Rockville, Maryland, United States of America, **2** Institute of Legal Medicine, Innsbruck Medical University, Innsbruck, Austria, **3** University of Florida, Gainesville, Florida, United States of America, **4** Department of Pure and Applied Chemistry, University of Strathclyde, Glasgow, United Kingdom, **5** Institute of Forensic Medicine, University of Oslo, Oslo, Norway

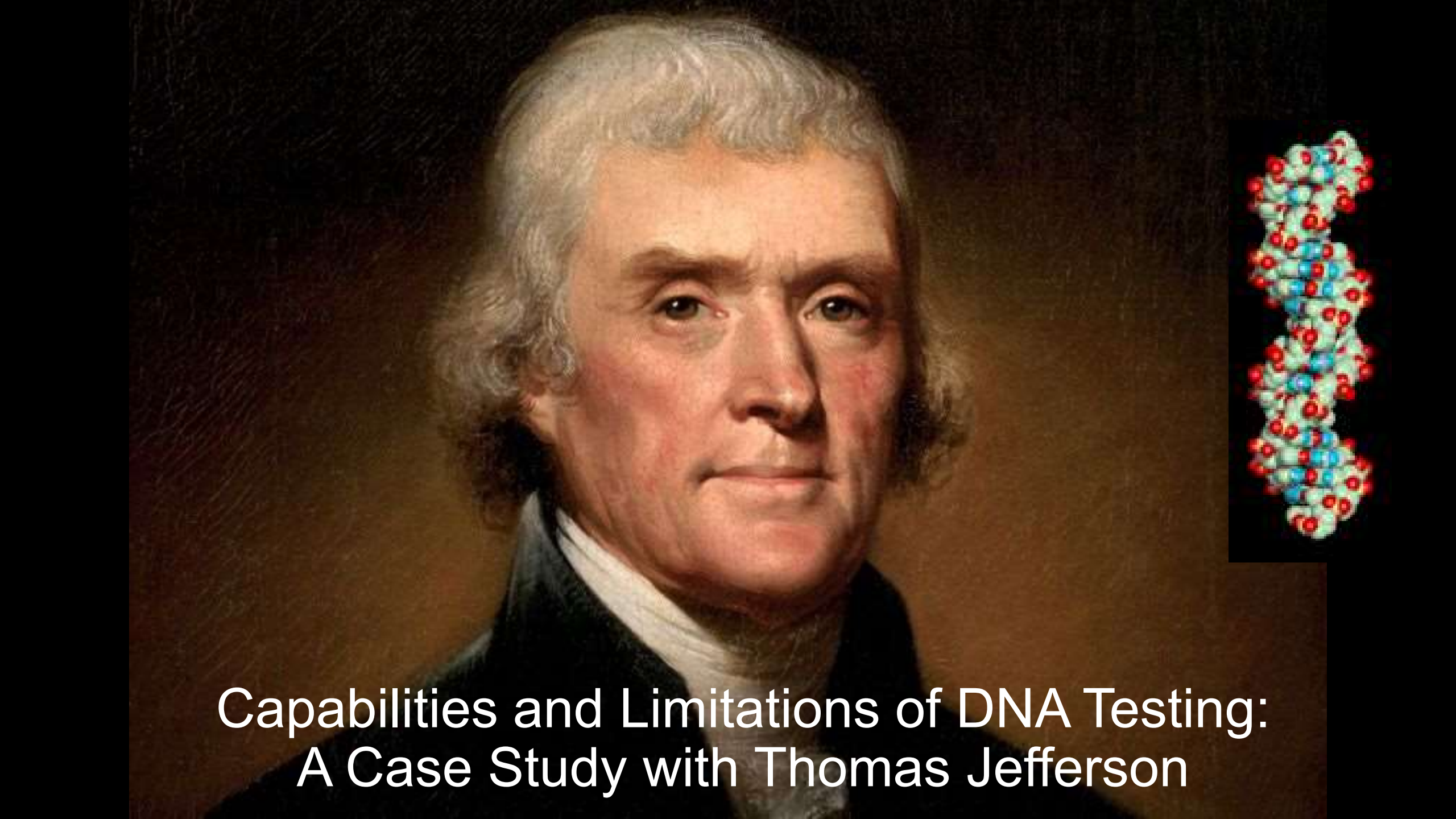
Genetic Genealogy

- Y-chromosome testing (since ~2000)
  - surname connections
  - FamilyTree DNA
- mtDNA testing (since ~2000)
  - Looking at human migration patterns through history (e.g., Genographic Project)
  - FamilyTree DNA, Oxford Ancestors

- Autosomal SNP testing (since ~2010)
  - Ancestry, 23andMe, My Heritage, FamilyTree DNA
  - >40 million people have contributed samples
  - Works well for connecting close cousins (up to 3rd and sometimes 4th cousins)

  - **Since Golden State Killer identified in 2018, >400 cold cases have been solved**

Forensic/Investigative Genetic Genealogy (FIGG, FGG, IGG)



Capabilities and Limitations of DNA Testing: A Case Study with Thomas Jefferson

http://dervan.caltech.edu/dervan.html

“Pay attention to your data!”

Peter Dervan

California Institute of Technology
Professor of Bioorganic Chemistry

October 4, 1993
UVA Department of Chemistry Seminar

As a young graduate student, I was taught an important lesson in a scientific seminar that I attended at the University of Virginia. A prominent bioorganic chemist from Cal Tech named Peter Dervan came to our chemistry department and spoke to a handful of students and professors. During the seminar, Professor Dervan shared a story where he and one of his graduate students made an important discovery that did not seem to make sense at the time. The experiments were repeated and again the results were reproduced but were unexpected. **Rather than throwing out the data because he could not explain it, Professor Dervan decided to examine it and consider it more carefully. Because he was attentive to detail, trusted in the ability of his student to correctly collect information from the experiment, and had a desire to pursue the truth wherever it might lead, a significant discovery was made, and knowledge was advanced in the area of nucleic acid chemistry.** While I frankly do not remember the details of the scientific advancement made, I came away from that seminar with a phrase that has impacted me ever since—“Pay attention to your data!”

Professor Dervan was a 2006 Recipient of the National Medal of Science
(his wife Jacqueline Barton received the National Medal of Science in 2010)

“In research, what’s important is not the answer, it’s finding the right questions.” – Peter Dervan
“At the beginning, it’s detective work--having a puzzle, a problem to solve.” – Jacqueline Barton

Background Information on Individuals of Interest

- **Thomas Jefferson (TJ)** (1743-1826): "Father of the University of Virginia"
  - With his wife Martha (Wayles) had six children before her death in 1782
  - Only two children, both daughters, lived to adulthood: Martha (Randolph) and Mary (Eppes)
- **Sally Hemings (SH)** (1773-1835)
  - An enslaved woman; half sister to Martha Wayles; to Paris in 1786 (at age 14) to assist TJ's daughters
  - Lived at Monticello until freed (along with other family members); died in Charlottesville; *gravesite unknown*
  - Had 6+ children: Tom (1790)?, Harriet I (1795-97), Beverly (1798-1822+), daughter? (1799), Harriet II (1801-1822+), **Madison** (1805-1877), **Eston** (1808-1856)
- **Thomas Woodson** (1790-1879)
  - His family claimed he was son of SH with TJ as his father
- **Peter Carr** (1770-1815) **& Samuel Carr** (1771-1855)
  - Nephews of Thomas Jefferson (sons of his sister); regular visitors to Monticello
  - Jefferson's grandchildren later claimed either were the father of SH's children
- **Randolph Jefferson** (1755-1815): younger brother of TJ; had 5 sons (4 of them ages 17 to 25 in 1807)
  - *Not considered in 1998 DNA study; would have the same Y-chromosome as TJ as would his 5 sons*
  - Lived ~20 miles from Monticello; would/could have visited when TJ was present

> James Callender 1802 claim: "[TJ] has kept, as his concubine, one of his own slaves. Her name is SALLY. The name of her eldest son is TOM…"
> (*The [Richmond] Recorder*, Sept 1, 1802)

Question of Interest: **Who fathered Sally Hemings' children? Can DNA data help answer?**

Nov 1998

scientific correspondence

Jefferson fathered slave's last child

There is a long-standing historical controversy over the question of US President Thomas Jefferson's paternity of the children of Sally Hemings, one of his slaves[1–4]. To throw some scientific light on the dispute, we have compared Y-chromosomal DNA haplotypes from male-line descendants of Field Jefferson, a paternal uncle of Thomas Jefferson, with those of male-line descendants of Thomas Woodson, Sally Hemings' putative first son, and of Eston Hemings Jefferson, her last son. The molecular findings fail to support the belief that Thomas Jefferson was Thomas Woodson's father, but provide evidence that he was the biological father of Eston Hemings Jefferson.

In 1802, President Thomas Jefferson was accused of having fathered a child, Tom, by Sally Hemings[5]. Tom was said to have been born in 1790, soon after Jefferson and Sally Hemings returned from France where he had been minister. Present-day members of the African–American Woodson family believe that Thomas Jefferson was the father of Thomas Woodson, whose name comes from his later owner[6]. No known documents support this view.

Sally Hemings had at least four more children. Her last son, Eston (born in 1808), is said to have borne a striking resemblance to Thomas Jefferson, and entered white society in Madison, Wisconsin, as Eston Hemings Jefferson. Although Eston's descendants believe that Thomas Jefferson was Eston's father, most Jefferson scholars give more credence to the oral tradition of the descendants of Martha Jefferson Randolph, the president's daughter. They believe that Sally Hemings' later children, including Eston, were fathered by either Samuel or Peter Carr, sons of Jefferson's sister, which would explain their resemblance to the president.

Because most of the Y chromosome is passed unchanged from father to son, apart from occasional mutations, DNA analysis of the Y chromosome can reveal whether or not individuals are likely to be male-line relatives. We therefore analysed DNA from the Y chromosomes of: five male-line descendants of two sons of the president's paternal uncle, Field Jefferson; five male-line descendants of two sons of Thomas Woodson; one male-line descendant of Eston Hemings Jefferson; and three male-line descendants of three sons of John Carr, grandfather of Samuel and Peter Carr (Fig. 1a). No Y-chromosome data were available from male-line descendants of President Thomas Jefferson because he had no surviving sons.

Seven bi-allelic markers (refs 7–12), eleven microsatellites (ref. 13) and the minisatellite MSY1 (ref. 14) were analysed (Fig. 1b). Four of the five descendants of Field Jefferson shared the same haplotype at all loci, and the fifth differed by only a single unit at one microsatellite locus, probably a mutation. This haplotype is rare in the population, where the average frequency of a microsatellite haplotype is about 1.5 per cent. Indeed, it has never been observed outside the Jefferson family, and it has not been found in 670 European men (more than 1,200 worldwide) typed with the microsatellites or 308 European men (690 worldwide) typed with MSY1.

Four of the five male-line descendants of Thomas Woodson shared a haplotype (with one MSY1 variant) that was not similar to the Y chromosome of Field Jefferson but was characteristic of Europeans. The fifth Woodson descendant had an entirely different haplotype, most often seen in sub-Saharan Africans, which indicates illegitimacy in the line after individual W42. In contrast, the descendant of Eston Hemings Jefferson did have the Field Jefferson haplotype. The haplotypes of two of the descendants of John Carr were identical; the third differed by one step at one microsatellite locus and by one step in the MSY1 code. The Carr haplotypes differed markedly from those of the descendants of Field Jefferson.

The simplest and most probable explanations for our molecular findings are that Thomas Jefferson, rather than one of the Carr brothers, was the father of Eston Hemings Jefferson, and that Thomas Woodson was not Thomas Jefferson's son. The frequency of the Jefferson haplotype is less than 0.1 per cent, a result that is at least 100 times more likely if the president was the father of Eston Hemings Jefferson than if someone unrelated was the father.

We cannot completely rule out other explanations of our findings based on illegitimacy in various lines of descent. For example, a male-line descendant of Field Jefferson could possibly have illegitimately fathered an ancestor of the presumed male-line descendant of Eston. But in the absence of historical evidence to support such possibilities, we consider them to be unlikely.

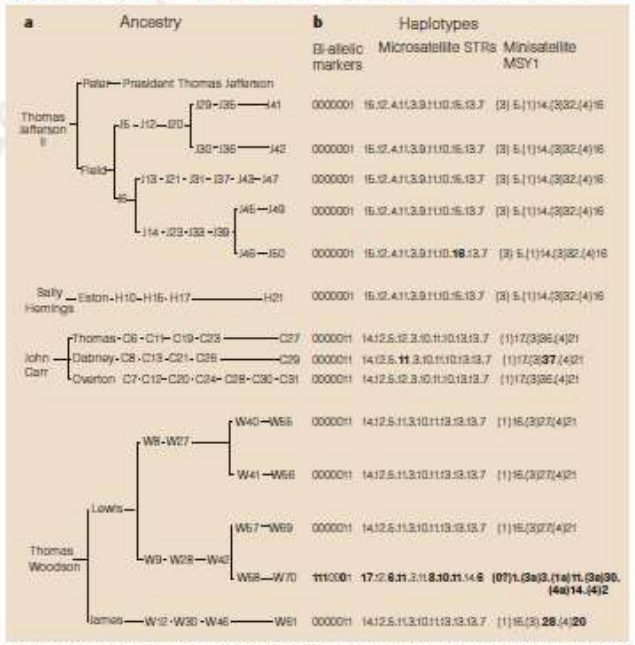


Figure 1 Male-line ancestry and haplotypes of participants. **a**, Ancestry. Numbers correspond to reference numbers and names in more detailed genealogical charts for each family. **b**, Haplotypes. Entries in bold highlight deviations from the usual patterns for the group of descendants. **Bi-allelic markers.** Order of loci: YAP-SRYm8299-sY81-LLY22g-Tat-92R7-SRYm1532. 0, ancestral state; 1, derived state. **Microsatellite short tandem repeats (STRs).** Order of loci: 19-388-389A-389B-389C-389D-390-391-392-393-dxys156y. The number of repeats at each locus is shown. **Minisatellite MSY1.** Each number in brackets represents the sequence type of the repeat unit; the number after it is the number of units with this sequence type. For example, j41 has 5 units of sequence type 3, 14 units of sequence type 1, 32 units of sequence type 3, and 16 units of sequence type 4.

Eugene A. Foster*, M. A. Jobling†, P. G. Taylor†, P. Donnelly‡, P. de Knijff§, Rene Mieremet§, T. Zerjal¶, C. Tyler-Smith¶

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13. Kayser, M. *et al. Int. J. Legal Med.* **110**, 125–133 (1997).
14. Jobling, M. A., Bouzekri, N. & Taylor, P. G. *Hum. Mol. Genet.* **7**, 643–653 (1998).

My Thoughts

- DNA testing was performed by **the top research laboratories in the world at the time for Y-chromosome analysis**
  - **Chris Tyler-Smith** then at Oxford (UK), recently retired from Sanger Institute
  - **Mark Jobling** at University of Leicester (UK)
  - **Peter de Knijff** at Leiden University (Holland)
- I have met each of these scientists at meetings over the last 24 years and we occasionally have contact via email
- **I trust the accuracy of their DNA test results** *(measurements, not necessarily the interpretation of their meaning)*
- There are limitations with Y-chromosome testing as **males from the same lineage usually cannot be resolved from one another** (unless a mutation occurs…)

Male Relatives Have the Same Y-Chromosome Results

17 Y-STR markers examined

My DNA results with 11 male relatives

	DYS 19	DYS 385a	DYS 385b	DYS 389I	DYS 389II	DYS 390	DYS 391	DYS 392	DYS 393	DYS 437	DYS 438	DYS 439	DYS 448	DYS 456	DYS 458	DYS 635	GATA -H4
Me	**14**	**11**	**15**	**13**	**29**	**24**	**11**	**13**	**13**	**15**	**12**	**13**	**19**	**17**	**18**	**23**	**12**
My **father**																	
My 1st **son**																	
My 2nd **son**																	
My 1st **brother**																	
My 2nd **brother**																	
My 3rd **brother**																	
My 4th **brother**																	
My 1st **nephew**																	
My 2nd **nephew**																	
My 3rd **nephew**																	
My 4th **nephew**																	

How Many Butler Y-chromosomes Are Out There?

Katherine **Butler** Gettings

NIST Applied Genetics Group
PhD George Washington University

John M. **Butler**

NIST Special Programs Office
PhD University of Virginia

Some interesting points:

1. **Katherine's father possesses an identical Yfiler 17-locus profile to John**
2. The first known Butler in John's lineage came to Virginia in the early 1700s – Katherine's family has been in Virginia since about the same time
3. Based on review of what they know from their family histories, they **cannot be closer than sixth or seventh cousins** (their 5th great-grandfathers differ)
4. **Potentially thousands of male Butlers have this same Yfiler haplotype** – or one very similar due to mutation at individual Y-STR loci
5. **A YHRD search** (conducted in Sept 2022) **results in a value of 2 out of 283,483 Yfiler profiles, which does not reflect the true haplotype frequency in the world** (and especially Virginia)

Some News Articles in November 1998

The New York Times

DNA Test Finds Evidence Of Jefferson Child by Slave

By Dinitia Smith and Nicholas Wade
November 1, 1998
Section 1, Page 1

DNA tests on the descendants of Thomas Jefferson's family and of Jefferson's young slave, Sally Hemings, **offer compelling evidence** that the nation's third President fathered at least one of her children, according to an article in the scientific journal *Nature*.

The report is based on blood samples collected by Eugene A. Foster of Charlottesville, Va., a retired Tufts University professor of pathology…

The Washington Post

Tests Link Jefferson, Slave's Son

By Leef Smith
Sunday, November 1, 1998
Page A01

Genetic testing shows that Thomas Jefferson **almost certainly fathered a child** with one of his slaves, Sally Hemings, according to scientists who argue that their results come as close as possible to solving one of history's most enduring and contentious mysteries...

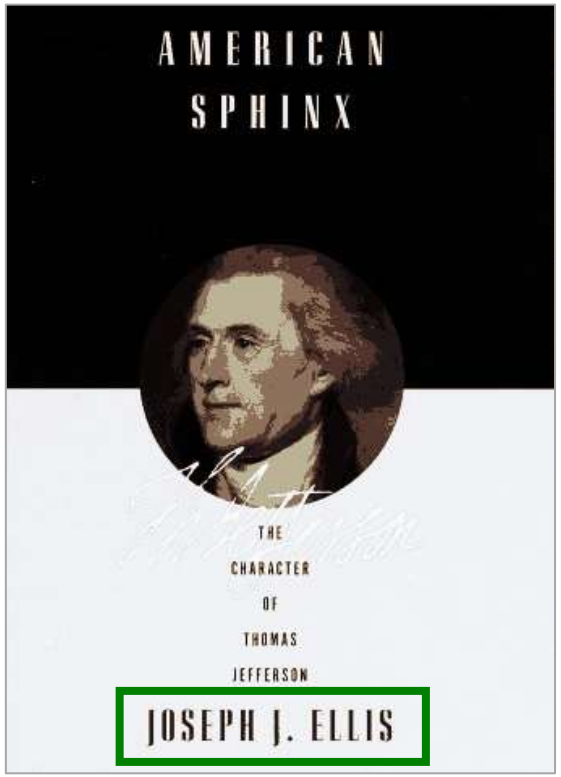
U.S. News & WORLD REPORT

When a Saint Becomes a Sinner

By Joseph J. Ellis
November 9, 1998, pp. 67-68

The DNA evidence proves that Jefferson had a long-term sexual relationship with his mulatto slave Sally. For the several hundred Hemings descendants, this news confirms the stories they have been passing through the generations. Among scholars, the acceptance of a Jefferson-Hemings liaison has been gaining ground…

Book published in 1996



"…after five years mulling over the huge cache of evidence that does exist on the thought and character of the historical Jefferson, I have concluded that **the likelihood of a liaison with Sally Hemings is remote**" (from pp. 303-307)

https://www.pbs.org/wgbh/pages/frontline/shows/jefferson/cron/1996sphinx.html

Nov 1998 in *Nature*

Founding father

Eric S. Lander and Joseph J. Ellis

Almost two hundred years ago Thomas Jefferson was alleged to have fathered a children by his slave Sally Hemings. The charges have remained controversial. Now, DNA analysis confirms that Jefferson was indeed the father of at least one of Hemings' children.

ny Jefferson's youngest daughter to Paris in 1786. There is no evidence of what transpired there, but Hemings returned to the United States with Jefferson in 1789, and she eventually bore at least five children, starting with Tom in 1790 and ending with Eston in 1808.

At least three pieces of evidence support a relationship between Jefferson and Hemings. First, several of the children bore a somes can be distinguished by the haplotype (set of specific variants) that they carry. Researchers from several laboratories have identified a collection of suitable markers from the Y chromosome over the past two years, and this collection is now fuelling an explosion in male-line genetic studies.

Foster *et al.*[1] examined a haplotype containing 19 polymorphic markers. Jefferson's haplotype (inferred from male-line descen-

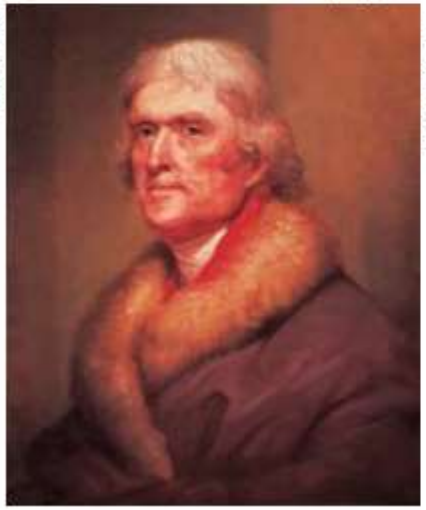


Figure 1 Thomas Jefferson (1743–1826), third president of the United States. DNA analysis by Foster *et al.*[1] shows that he fathered at least one child by his slave, Sally Hemings.

the circumstantial evidence, it seems to seal the case that Jefferson was Eston Hemings' father.

Interestingly, Jefferson's haplotype does not match male descendants of Sally's first son, Tom Woodson. The simplest explanation is that Jefferson was not Tom's father. An alternative explanation would require non-paternities among Tom's offspring. The jury remains out with respect to Sally's other children, but the burden of proof has clearly shifted.

Nothing in Foster and colleagues' study, and nothing in the vast historical literature, sheds any light on the character of the relationship between Jefferson and Sally Hemings. Was it, as his contemporary critics charged, a tale of lust and rape? Was it, as several twentieth-century scholars and novelists have suggested, a love story rooted in mutual affection? Or was it something in-between? These questions are open to endless interpretation but, in a broader sense, the new findings give blacks and whites alike an opportunity to confront a largely secret, shared history.

Politically, the Thomas Jefferson verdict is likely to figure in upcoming impeachment hearings on William Jefferson Clinton's sexual indiscretions, in which DNA testing has also played a role. The parallels are hardly perfect, but some are striking. Both 'improper' relationships involved women about 28 years younger — although there is a world of difference between a slave and master at the close of the eighteenth century, and a White House intern and a married man at the end

racial affair now personalizes this issue, while adding a dimension of hypocrisy.

Massachusetts 01075, USA.

1. Foster, E. A. *et al.* *Nature* **396**, 27–28 (1998).

SCIENCE
8 Jan 1999
Vol 283, Issue 5399
pp. 153-155
https://www.science.org/doi/10.1126/science.283.5399.153a

"Contrary to headlines that splashed across the country in November, there is no conclusive proof that former U.S. President Thomas Jefferson fathered an illegitimate child by his slave Sally Hemings. At least five of his family members are candidates for paternity of Sally's child, researchers admit in a letter in tomorrow's issue of *Nature*."

By Eliot Marshall | Jan. 6, 1999 , 7:00 PM

https://www.science.org/content/article/thomas-jefferson-hook

Nov 1998 in *Nature*

scientific correspondence

Jefferson fathered slave's last child

There is a long-standing historical controversy over the question of US President Thomas Jefferson's paternity of the children of Sally Hemings, one of his slaves[1–4]. To throw some scientific light on the dispute, we have compared Y-chromosomal DNA haplotypes from male-line descendants of Field Jefferson, a paternal uncle of Thomas Jefferson, with those of male-line descendants of Thomas Woodson, Sally Hemings' putative first son, and of Eston Hemings Jefferson, her last son. The molecular findings fail to support the belief that Thomas Jefferson was Thomas Woodson's father, but provide evidence that he was the biological father of Eston Hemings Jefferson.

In 1802, President Thomas Jefferson was accused of having fathered a child, Tom, by Sally Hemings[5]. Tom was said to have been born in 1790, soon after Jefferson and Sally Hemings returned from France where he had been minister. Present-day members of the African–American Woodson family believe that Thomas Jefferson was the father of Thomas Woodson, whose name comes from his later owner[6]. No known documents support this view.

Sally Hemings had at least four more children. Her last son, Eston (born in 1808), is said to have borne a striking resemblance to Thomas Jefferson, and entered white society in Madison, Wisconsin, as Eston Hemings Jefferson. Although Eston's descendants believe that Thomas Jefferson was Eston's father, most Jefferson scholars give more credence to the oral tradition of the descendants of Martha Jefferson Randolph, the president's daughter. They believe that Sally Hemings' later children, including Eston, were fathered by either Samuel or Peter Carr, sons of Jefferson's sister, which would explain their resemblance to the president.

Because most of the Y chromosome is passed unchanged from father to son, apart from occasional mutations, DNA analysis of the Y chromosome can reveal whether or not individuals are likely to be male-line relatives. We therefore analysed DNA from the Y chromosomes of: five male-line descendants of two sons of the president's paternal uncle, Field Jefferson; five male-line descendants of two sons of Thomas Woodson; one male-line descendant of Eston Hemings Jefferson; and three male-line descendants of three sons of John Carr, grandfather of Samuel and Peter Carr (Fig. 1a). No Y-chromosome data were available from male-line descendants of President Thomas Jefferson because he had no surviving sons.

Seven bi-allelic markers (refs 7–12), eleven microsatellites (ref. 13) and the minisatellite MSY1 (ref. 14) were analysed (Fig. 1b). Four of the five descendants of Field Jefferson shared the same haplotype at all loci, and the fifth differed by only a single unit at one microsatellite locus, probably a mutation. This haplotype is rare in the population, where the average frequency of a microsatellite haplotype is about 1.5 per cent. Indeed, it has never been observed outside the Jefferson family, and it has not been found in 670 European men (more than 1,200 worldwide) typed with the microsatellites or 308 European men (690 worldwide) typed with MSY1.

Four of the five male-line descendants of Thomas Woodson shared a haplotype (with one MSY1 variant) that was not similar to the Y chromosome of Field Jefferson but was characteristic of Europeans. The fifth Woodson descendant had an entirely different haplotype, most often seen in sub-Saharan Africans, which indicates illegitimacy in the line after individual W42. In contrast, the descendant of Eston Hemings Jefferson did have the Field Jefferson haplotype. The haplotypes of two of the descendants of John Carr were identical; the third differed by one step at one microsatellite locus and by one step in the MSY1 code. The Carr haplotypes differed markedly from those of the descendants of Field Jefferson.

The simplest and most probable explanations for our molecular findings are that Thomas Jefferson, rather than one of the Carr brothers, was the father of Eston Hemings Jefferson, and that Thomas Woodson was not Thomas Jefferson's son. The frequency of the Jefferson haplotype is less than 0.1 per cent, a result that is at least 100 times more likely if the president was the father of Eston Hemings Jefferson than if someone unrelated was the father.

We cannot completely rule out other explanations of our findings based on illegitimacy in various lines of descent. For example, a male-line descendant of Field

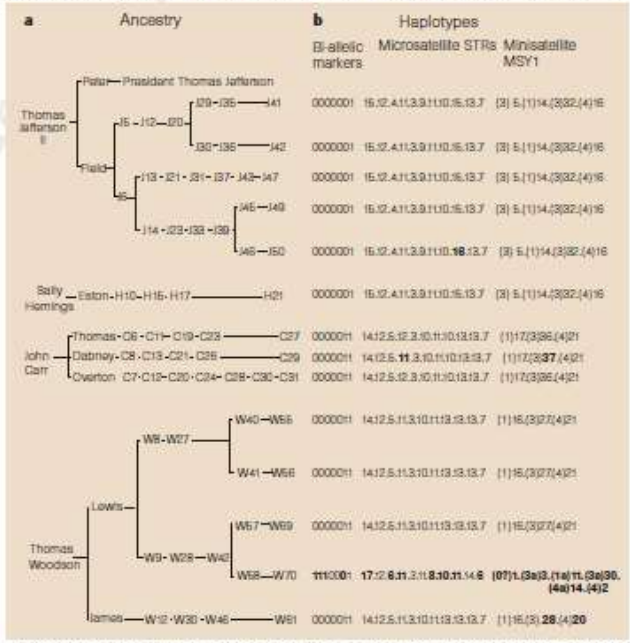


Figure 1 Male-line ancestry and haplotypes of participants. **a**, Ancestry. Numbers correspond to reference numbers and names in more detailed genealogical charts for each family. **b**, Haplotypes. Entries in bold highlight deviations from the usual patterns for the group of descendants. **Bi-allelic markers.** Order of loci: YAP-SRYm8299-sY81-LLY22g-Tat-92R7-SRYm1532. 0, ancestral state; 1, derived state. **Microsatellite short tandem repeats (STRs).** Order of loci: 19-388-389A-389B-389C-389D-390-391-392-393-dxys156y. The number of repeats at each locus is shown. **Minisatellite MSY1.** Each number in brackets represents the sequence type of the repeat unit; the number after it is the number of units with this sequence type. For example, J41 has 5 units of sequence type 3, 14 units of sequence type 1, 32 units of sequence type 3, and 16 units of sequence type 4.

Follow-Up Articles
(not discussed by media)

Jefferson could possibly have illegitimately fathered an ancestor of the presumed male-line descendant of Eston. But in the absence of historical evidence to support such possibilities, we consider them to be unlikely.

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14. Jobling, M. A., Bouzekri, N. & Taylor, P. G. *Hum. Mol. Genet.* **7**, 643–653 (1998).

scientific correspondence

Jan 1999 in *Nature*

The Thomas Jefferson paternity case

The DNA analysis of Y-chromosome haplotypes used by Foster *et al.*[1] to evaluate Thomas Jefferson's alleged paternity of Eston Hemings Jefferson, the last child of his slave Sally Hemings, is impressive. However, the authors did not consider all the data at hand in interpreting their results.

No mention was made of Thomas Jefferson's brother Randolph (1757–1815), or of his five sons[2,3]. Sons of Sally Hemings conceived by Randolph (or by one of his sons) would produce a Y-chromosome analysis identical to that described by Foster *et al.* Further collaborative data (for example, the whereabouts of any of those who might have been involved at conception) are needed to confirm that Jefferson did indeed father his slave's last child, as claimed in the title. We know Thomas Jefferson was there, but how about Randolph Jefferson and his sons?

David M. Abbey
Health Science Center, University of Colorado, 1512 Teakwood Court, Fort Collins, Colorado 80525, USA
e-mail: dabbey1000@aol.com

If the data of Foster *et al.* are accurate, then any male ancestor in Thomas Jefferson's line, white or black, could have fathered Eston Hemings. Plantations were inbred communities, and the mixing of racial types was probably common. As slave families were passed as property to the owner's offspring along with land and other property, it is possible that Thomas Jefferson's father, grandfather or paternal uncles fathered a male slave whose line later impregnated another slave, in this case Sally Hemings. Sally herself was a light mulatto, known even at that time to be Thomas Jefferson's wife's half sister[3,4].

Gary Davis
Evanston Hospital, 2650 Ridge Avenue, Evanston, Illinois 60201, USA

Foster et al. *reply* — It is true that men of Randolph Jefferson's family could have fathered Sally Hemings' later children. Space constraints prevented us from expanding on alternative interpretations of our DNA analysis, including the interesting one proposed by Davis. The title assigned to our study was misleading in that it represented only the simplest explanation of our molecular findings: namely, that Thomas Jefferson, rather than one of the Carr brothers, was likely to have been the father of Eston Hemings Jefferson.

It had been suggested to us earlier (by Herbert Barger, who also helped to recruit the descendants of Field Jefferson who participated in our study) that Isham Jefferson, son of Thomas Jefferson's brother Randolph, might have been the father of one or more of Sally Hemings' children. Barger's proposal was based on a statement[5] that Isham was reared by Thomas Jefferson; he suggested that Isham could have been at Monticello or at Snowden (Snowden was across the James River from Scottsville, which is about 20 miles from Monticello) when Eston Hemings was conceived. But it is not known for certain that Isham was at Monticello at that time, whereas it is documented that Thomas Jefferson was. From the historical knowledge we have, we cannot conclude that Isham, or any other member of the Jefferson family, was as likely as Thomas Jefferson to have fathered Eston Hemings.

We know from the historical and the DNA data that Thomas Jefferson can neither be definitely excluded nor solely implicated in the paternity of illegitimate children with his slave Sally Hemings. When we embarked on this study, we knew that the results could not be conclusive, but we hoped to obtain some objective data that would tilt the weight of evidence in one direction or another. We think we have provided such data and that the modest, probabilistic interpretations we have made are tenable at present.

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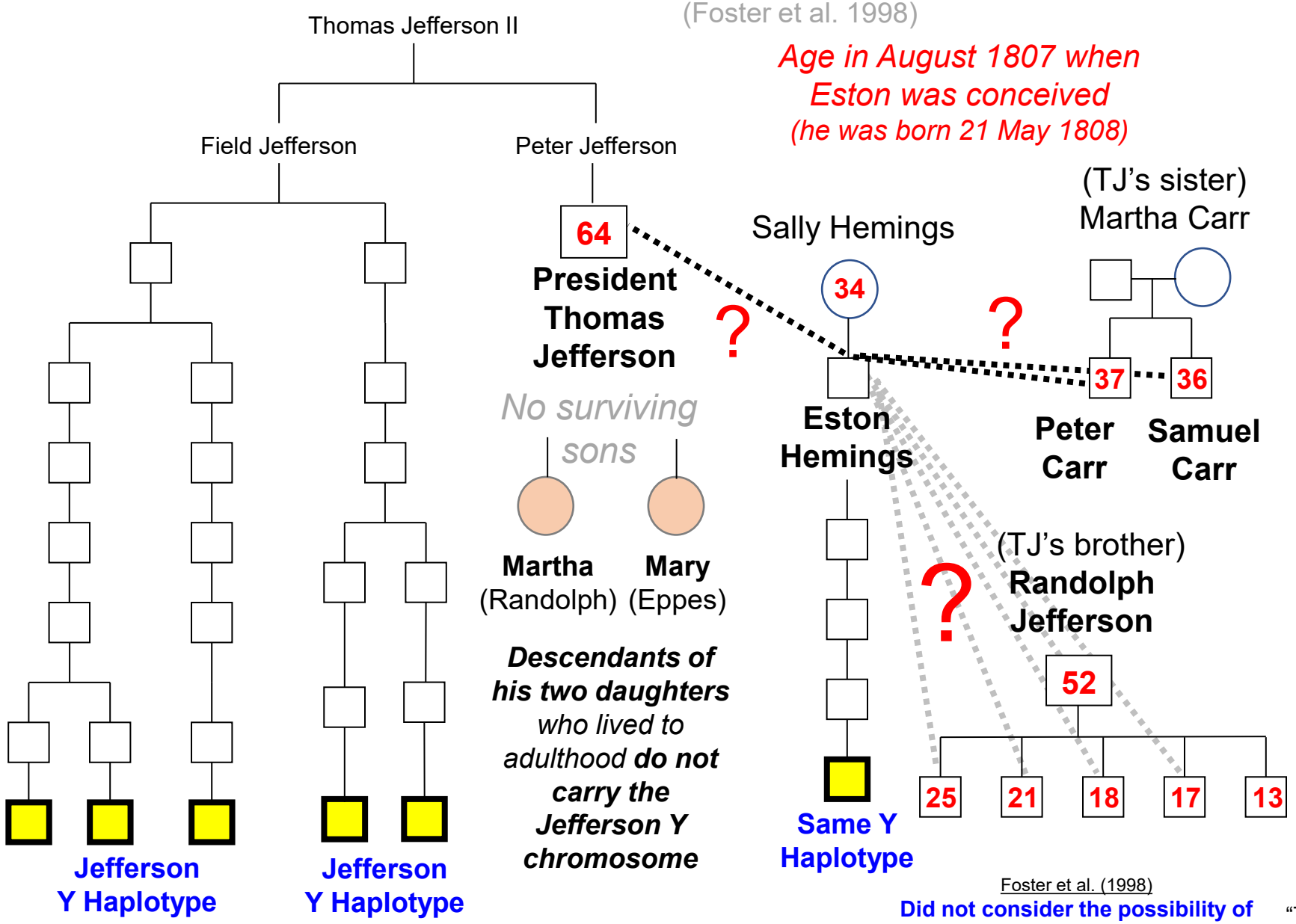
2. Mayo, B. & Bear, J. A. Jr *Thomas Jefferson and his Unknown Brother* (Univ. Press of Virginia, Charlottesville, 1981).
3. Brodie, F. M. *Thomas Jefferson: An Intimate History* (Norton, New York, 1974).
4. Randall, W. S. *Thomas Jefferson: A Life* (Holt, New York, 1993).
5. *History of Todd County, Kentucky* (1884).

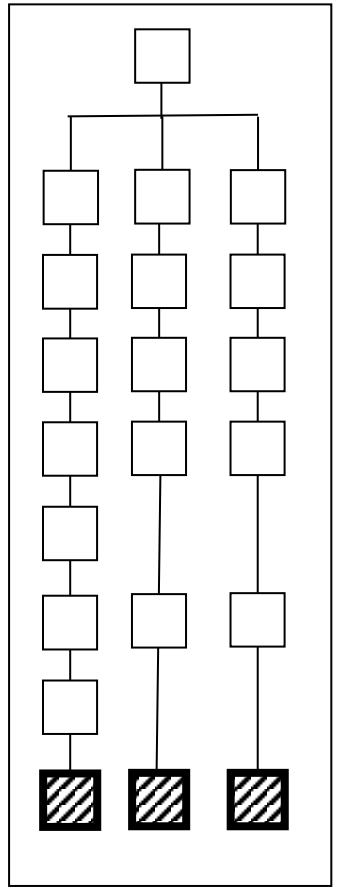
Y-chromosome Study with Jefferson-Hemings DNA

(Foster et al. 1998)



Foster et al. (1998)
Did not consider the possibility of Randolph or his sons who would have the same Y chromosome

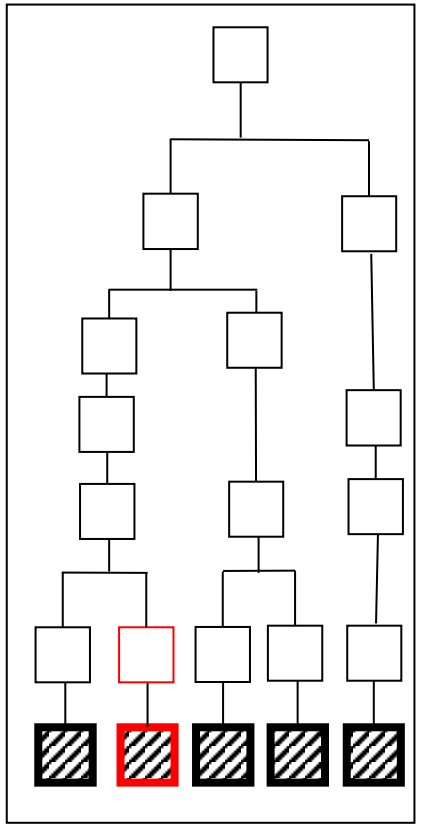
Examined multiple descendants of **John Carr** (Peter and Samuel's paternal grandfather)



Different Y Haplotype

Foster et al. (1998)
"The Carr haplotypes differed markedly from those of the descendants of Field Jefferson."

Examined multiple descendants of **Thomas Woodson** (from TJ and SH by oral family history)



Different Y Haplotype

Foster et al. (1998)
"…Thomas Woodson was not Thomas Jefferson's son."

Using living relatives prevents exhumations (better DNA quality); multiple lines verify lineage haplotypes

Thomas Jefferson

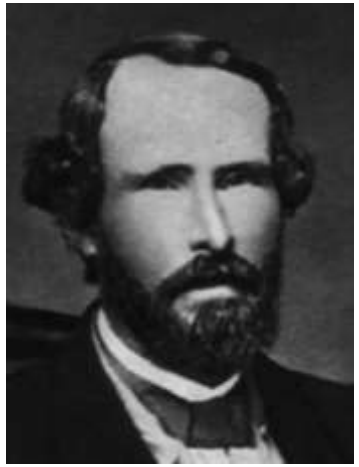
https://www.monticello.org/visit/tips-for-visiting/jefferson-s-gravesite/

Monticello Graveyard
Charlottesville, Virginia

Q ????? K

(1743-1826)

Having Randolph's DNA would be helpful *to fully exclude* him vs. his older brother Thomas



(1808-1856)

Willingness of families to exhume remains?

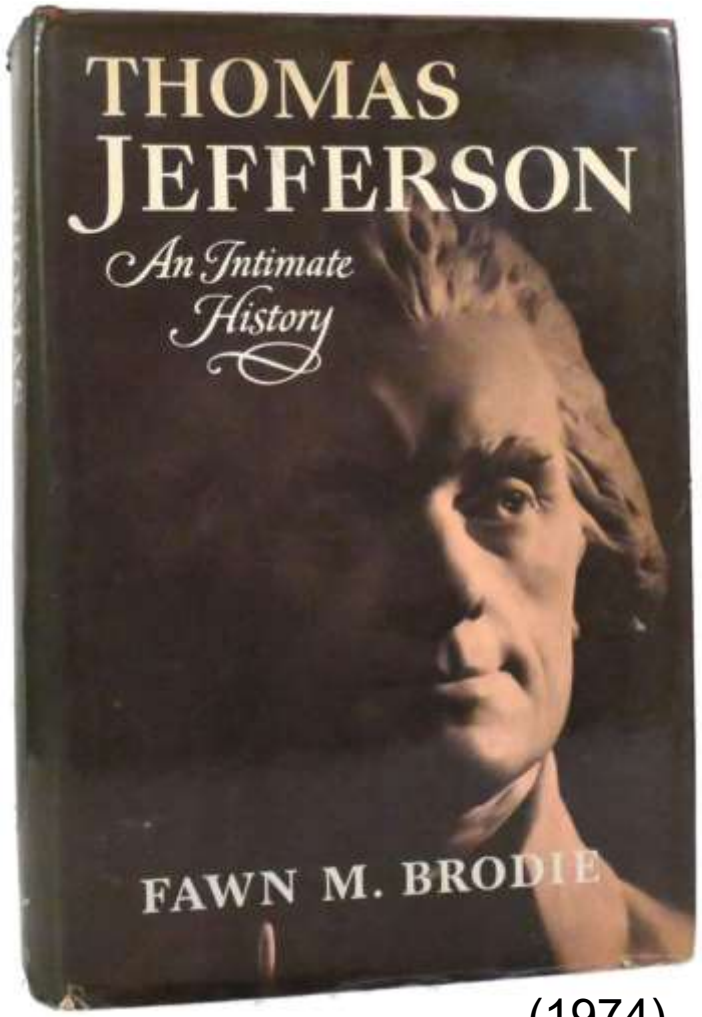
Conditions of almost 200-year-old remains?

Eston Hemings

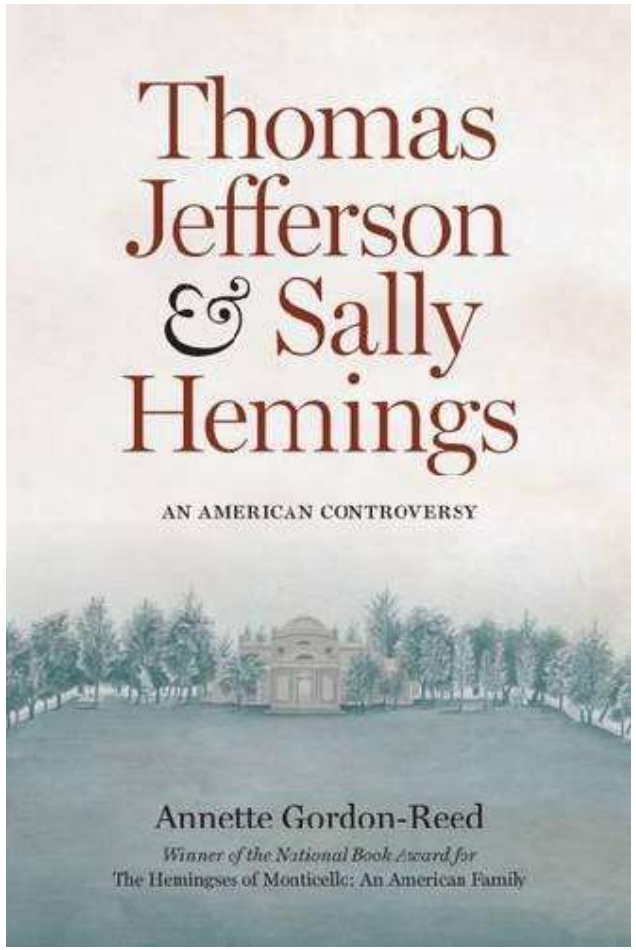
https://www.findagrave.com/memorial/10606815/eston-hemings-jefferson

Forest Hill Cemetery
Madison, Dane County, Wisconsin
PLOT Section 3, Lot 018, Grave 3

Advocating for a Jefferson-Hemings Relationship



(1974)



(1997, 1998)

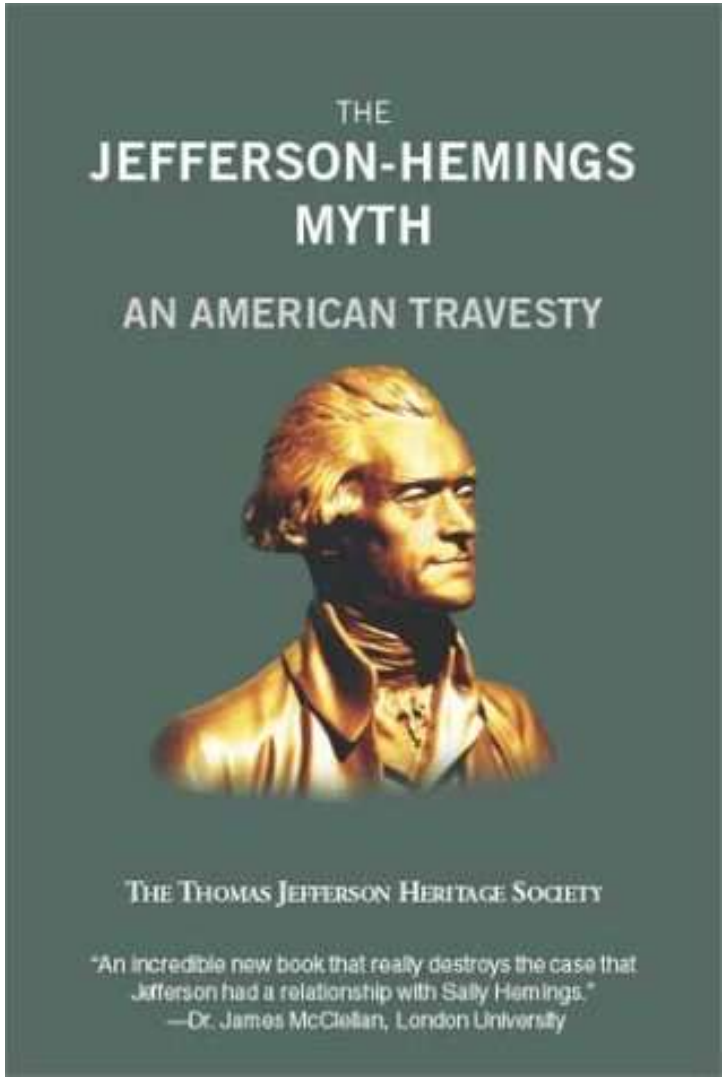
From Author's Note (pages x and xi) added in 1998 paperbook edition after DNA results became available:

"The DNA test does not prove that the descendant of Eston Hemings was a direct descendant of Thomas Jefferson. It does establish that he is linked to the Jefferson family, and not to the Carr family."

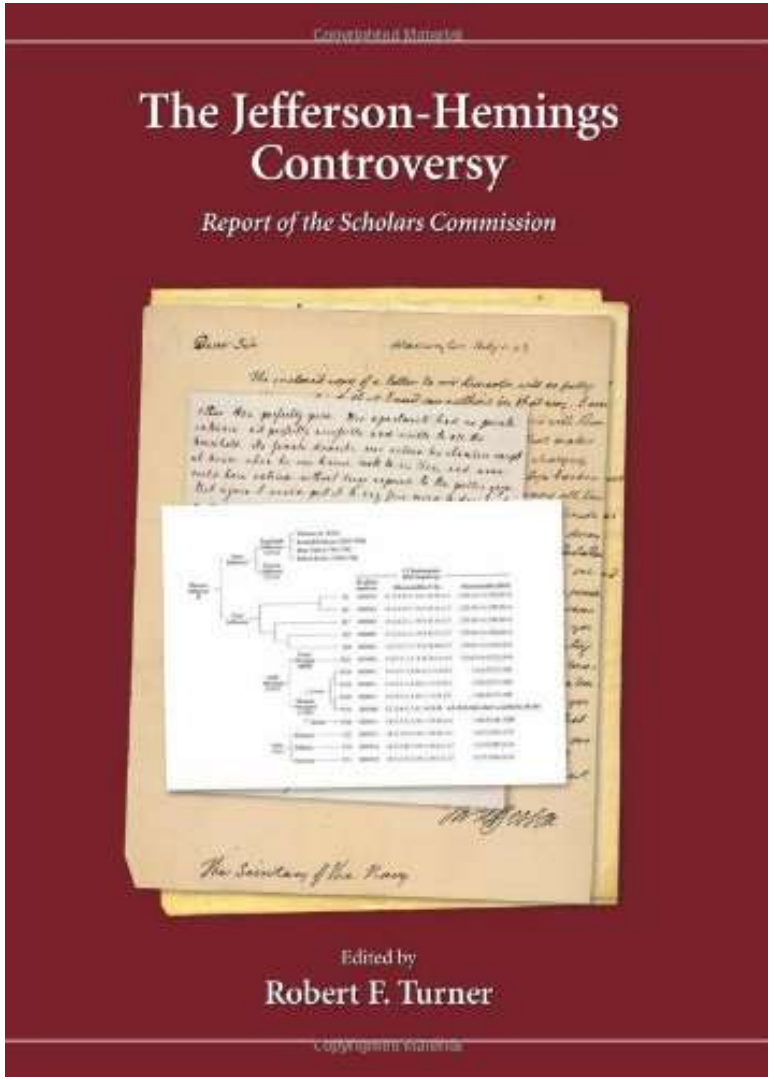
"Make no mistake, DNA is important, but it is not all we have to go on. It is not all we should go on. The scientific evidence necessarily must be read along with the existing historical evidence."

"If the question is framed as 'Do we have scientific proof that all of Sally Heming's children were fathered by Thomas Jefferson,' **the answer is no.** That is not, I think, the relevant question for historians. We don't have scientific proof of Jefferson's paternity of anyone."

Arguing Against a Jefferson-Hemings Relationship



(2001)



Original report in April 2001 (2011)

13-member Scholars Commission

From summary on page 3

"The question of whether Thomas Jefferson fathered one or more children by his slave Sally Hemings is an issue about which honorable people can and do disagree. After a careful review of all of the evidence, **the commission agrees unanimously that the allegation is by no means proven**; and **we find it regrettable that public confusion about the 1998 DNA testing and other evidence has misled many people**…"

Does DNA Prove that Jefferson Fathered Sally Hemings Children? No!

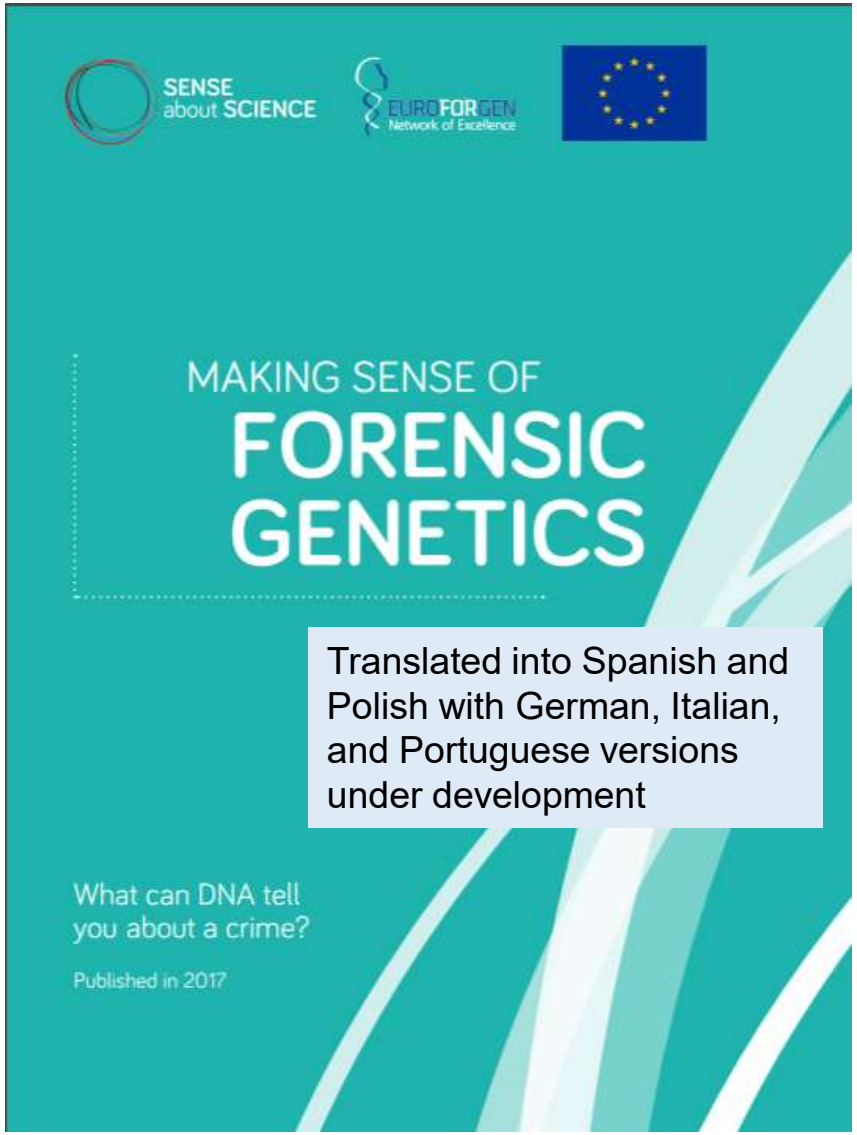
http://www.pbs.org/wgbh/nova/israel/familyjefferson.html

- Based on the Y-chromosome results, **any other male Jefferson at the time could be the father of Eston Hemings**
  - Known to be at least 25 male Jeffersons living in Central Virginia
  - Thomas Jefferson's younger brother Randolph or one of his sons are possibilities

- The 1998 DNA testing showed that
  - Descendants of Peter and Samuel Carr (TJ's nephews) cannot be associated with a descendant of Eston Hemings – *invalidating this claim of TJ's descendants*
  - Descendants of Thomas Woodson (oral history as TJ and SH first child) cannot be associated with the Jefferson male lineage – *invalidating this claim of TW's descendants*

Making Sense of Forensic Genetics (2017)

concepts clearly explained in 40 pages



Translated into Spanish and Polish with German, Italian, and Portuguese versions under development

- Developed by European Forensic Genetics Network of Excellence (EuroForGen-NoE) and **published with Sense about Science**
- **Free PDF file** available for download https://senseaboutscience.org/wp-content/uploads/2017/01/making-sense-of-forensic-genetics.pdf
- *Final point made*: "As DNA profiling continues to grow more sensitive, and it is used in more investigations, **the need for accurate communication between scientists and nonscientists only grows** - both **to ensure that their expectations of the technology are realistic, and its limits are properly understood**…"

Thank you for your attention!

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U.S. DEPARTMENT OF COMMERCE

https://www.nist.gov/topics/forensic-science

Questions?