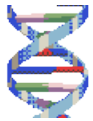
American Academy of Forensic Sciences
Orlando, FL
February 20, 2015

ORLANDO 2015

Why DNA Interpretation Has Become More Challenging in Recent Years

John M. Butler, Ph.D.
NIST Fellow & Special Assistant to the Director for Forensic Science
National Institute of Standards and Technology
Gaithersburg, Maryland

NIST FORENSIC SCIENCES



NIST

Acknowledgment and Disclaimers

I will quote from my recent book entitled “Advanced Topics in Forensic DNA Typing: Interpretation” (Elsevier, 2015). I do not receive any royalties for this book. Completing this book was part of my job last year at NIST.

Although I chaired the SWGDAM Mixture Committee that produced the 2010 STR Interpretation Guidelines**, I cannot speak for or on behalf of the Scientific Working Group on DNA Analysis Methods.**

I have been fortunate to have had discussions with numerous scientists on interpretation issues including Mike Coble, Bruce Heidebrecht, Robin Cotton, Charlotte Word, Catherine Grgicak, Peter Gill, Ian Evett …

Points of view are mine and do not necessarily represent the official position or policies of the US Department of Justice or the National Institute of Standards and Technology.

Certain commercial equipment, instruments and materials are identified in order to specify experimental procedures as completely as possible. In no case does such identification imply a recommendation or endorsement by the National Institute of Standards and Technology nor does it imply that any of the materials, instruments or equipment identified are necessarily the best available for the purpose.

Steps in Forensic DNA Analysis

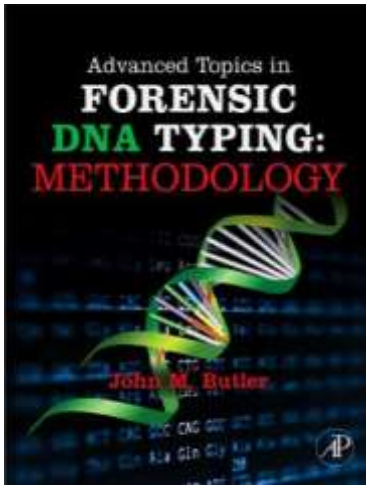
Understanding Results Obtained & Sharing Them

Gathering the Data

Interpretation

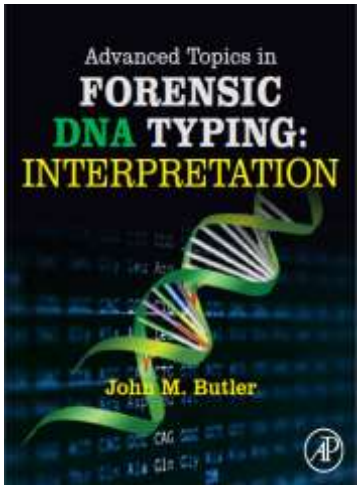
>1300 pages of information with >5000 references cited in these two books

Advanced Topics: Methodology



August 2011

Advanced Topics: Interpretation



October 2014

Ian Evett on Interpretation

"The crucial element that the scientist brings to any case is the *interpretation* of those observations. This is the heart of forensic science: it is where the scientist adds value to the process."

Evett, I.W., et al. (2000). The impact of the principles of evidence interpretation on the structure and content of statements. *Science & Justice, 40,* 233-239.

Information from Chapter 7 of my New Book

Advanced Topics in Forensic DNA Typing: Interpretation

CHAPTER

7

Low-Level DNA and Complex Mixtures

"The limits of each DNA typing procedure should be understood, especially when the DNA sample is small, is a mixture of DNA from multiple sources, or is contaminated with interfering substances."

NRC I, 1992, p. 8

"For the complex DNA profile, there is no predominant or overarching standard interpretation method."

Peter Gill (Gill et al. 2012, report to the UK Forensic Science Regulator, p. 18)

"The limits of each DNA typing procedure should be understood, especially when the DNA sample is small, is a mixture of DNA from multiple sources…" (NRC I, 1992, p. 8)

Butler, J.M. (2015) *Advanced Topics in Forensic DNA Typing: Interpretation* (Elsevier Academic Press: San Diego), pp. 159-182

Professor Peter Gill

June 2014; 100 pages

Concerns have been Raised over Potential for DNA Contamination

Previous articles by Peter Gill on this topic:

- Gill, P. (1997). The utility of 'substrate controls' in relation to 'contamination'. *Forensic Science International,* 85(2):105-111.
- Gill, P., & Kirkham, A. (2004). Development of a simulation model to assess the impact of contamination in casework using STRs. *Journal of Forensic Sciences,* 49(3): 485-491.
- Gill, P., et al. (2010). Manufacturer contamination of disposable plastic-ware and other reagents—an agreed position statement by ENFSI, SWGDAM and BSAG. *Forensic Science International: Genetics,* 4(4): 269-270.

Discusses the Amanda Knox case DNA results

5 Reasons that DNA Results Are Becoming More Challenging to Interpret

1. **More sensitive DNA test results**
2. **More touch evidence samples** that are poor-quality, low-template, complex mixtures
3. **More options exist** for statistical approaches involving probabilistic genotyping software
4. **Many laboratories are not prepared** to cope with complex mixtures
5. **More loci being added** because of the large number of samples in DNA databases

More Sensitive Assays and Instruments

- **Superb sensitivity is available** with DNA amplification using the polymerase chain reaction and laser-induced fluorescence detection with capillary electrophoresis

- Since 2007 (beginning with the release of the MiniFiler STR kit), **improved buffers and enzymes** have been used to boost DNA sensitivities in all STR kits
  - In 2010 the ABI 3500 Genetic Analyzer was released with 4X signal over the previous ABI 3100 and ABI 310 instruments
  - Energy-transfer dyes are used with some of the STR kits
  - Some labs increase the sensitivity dial with additional PCR cycles

- **So what is wrong with have improved sensitivity?**

Improved Sensitivity is a Two-Edged Sword

"As sensitivity of DNA typing improves, laboratories' abilities to examine smaller samples increases. This improved sensitivity is a two-edged sword. **With greater capabilities comes greater responsibilities to report meaningful results.** Given the possibility of DNA contamination and secondary or even tertiary transfer in some instances, **does the presence of a single cell (or even a few cells) in an evidentiary sample truly have meaning?**…"

Butler, J.M. (2015) *Advanced Topics in Forensic DNA Typing: Interpretation* (Elsevier Academic Press: San Diego), p. 458

Ian Evett and Colleagues' **Case Assessment and Interpretation:**

Hierarchies of Propositions

TABLE 16.2 Hierarchical Levels of Propositions Originally Developed by the UK Forensi 1998a, 1998b, Evett et al. 2000a, 2000b, Gill 2001)

Hierarchy Levels		Propositions	Decision Maker
Level III	Offense	Supplies the probability that a suspect has committed a criminal offense	Responsibility of the jury or judge
Level II	Activity	Informs regarding the kinds of activities which may have produced the forensic evidence	Jury or possibly scientist if given adequate case circumstances
Level I	Source	Addresses the source of the sample	Scientist
Sub-level I	Sub-source	With low amounts of DNA, the scientist may not be able to infer how the DNA arrived at the site where the DNA sample was collected	Scientist

Butler, J.M. (2015) *Advanced Topics in Forensic DNA Typing: Interpretation* (Elsevier Academic Press: San Diego), p. 458

Spinal Tap Volume Dial That Goes to 11 (on a scale of 1 to 10)

A volume dial or knob turned all the way to 11 surpassing and exceeding the normal maximum sound on a speaker or amplifier, resembling a famous scene from a mock rock documentary.

“…these dials go to eleven…”

http://www.dreamstime.com/stock-photos-one-goes-to-11-volume-dial-knob-image19921033

http://www.founditemclothing.com/t-shirts/gfx/spinal-tap-skeleton-shirt/spinal-tap-cap-2.jpg

More Touch Evidence Samples

https://www.ncjrs.gov/pdffiles1/nij/grants/222318.pdf

The DNA Field Experiment:
Cost-Effectiveness Analysis of the Use of DNA in the Investigation of High-Volume Crimes

John K. Roman
Shannon Reid
Jay Reid
Aaron Chalfin
William Adams
Carly Knight

Expanded DNA testing for burglary cases

NIJ April 2008 Research Report

http://www.nij.gov/journals/261/pages/dna-solves-property-crimes.aspx

DNA Solves Property Crimes (But Are We Ready for That?)
by Nancy Ritter

NIJ Journal October 2008 (vol. 261, pp. 2-12)

- **More poor-quality samples are being submitted**
  - Samples with <100 pg of DNA submitted in Belgium:
    19% (2004) → 45% (2008)
    (Michel 2009 FSIGSS 2:542-543)
- AAFS 2014 presentations showed poor success rates
  - NYC (**A110**): **only 10% of >9,500 touch evidence swabs from 2007 to 2011 produced usable DNA results**
  - Allegheny County (**A114**): examined touch DNA items processed from 2008 to 2013 across different evidence types (e.g., 6 of 56 car door handles yielded "resolvable profiles")

New Options Exist for Statistical Analysis

- Increase in approaches to try and cope with potential allele dropout → number of **probabilistic genotyping** methods have grown since Balding & Buckleton 2009 article

- Many possible choices for **probabilistic genotyping software** with commercial interests at stake

Balding, D.J. & Buckleton, J. (2009) Interpreting low template DNA profiles. *Forensic Sci. Int. Genet.* 4(1):1-10.

Gill P, Whitaker J, Flaxman C, Brown N, Buckleton J. (2000) An investigation of the rigor of interpretation rules for STRs derived from less than 100 pg of DNA. *Forensic Sci. Int.* 112(1):17-40.

TABLE 13.1 **Probabilistic Genotyping Software Programs (as of March 2014)**

Program Name	Type	Creator(s)	Availability
LRmix	Discrete (semi-continuous)	Hinda Haned & Peter Gill	Open-source https://sites.google.com/site/forensicdnastatistics/PCR-simulation/lrmix
Lab Retriever	Discrete (semi-continuous)	Developed by David Balding and maintained by Norah Rudin and colleagues	Open-source http://www.scieg.org/lab_retriever.html
likeLTD	Discrete (semi-continuous)	David Balding	Open-source https://sites.google.com/site/baldingstatisticalgenetics/software/likeltd-r-forensic-dna-r-code
FST	Discrete (semi-continuous)	Adele Mitchell	Proprietary to the NYC OCME Forensic Biology Laboratory
Armed Xpert	Discrete (semi-continuous)	Developed by USACIL and maintained and improved by NicheVision	Commercial product http://www.armedxpert.com/
TrueAllele	Fully-continuous	Mark Perlin	Commercial product http://www.cybgen.com/
STRmix	Fully-continuous	Duncan Taylor, Jo-Anne Bright, John Buckleton	Commercial product http://strmix.esr.cri.nz/
DNA View Mixture Solution	Fully-continuous	Charles Brenner	Commercial product http://dna-view.com/

Discrete (semi-continuous) methods use only the allele information in conjunction with probabilities of drop-out and drop-in.
Fully-continuous methods use peak height data and other parameters in addition to the allele information.

Butler, J.M. (2015) *Advanced Topics in Forensic DNA Typing: Interpretation* (Elsevier Academic Press: San Diego), p. 341

Probabilistic Genotyping via Modeling Simulations

Mathematical Modeling of the Data

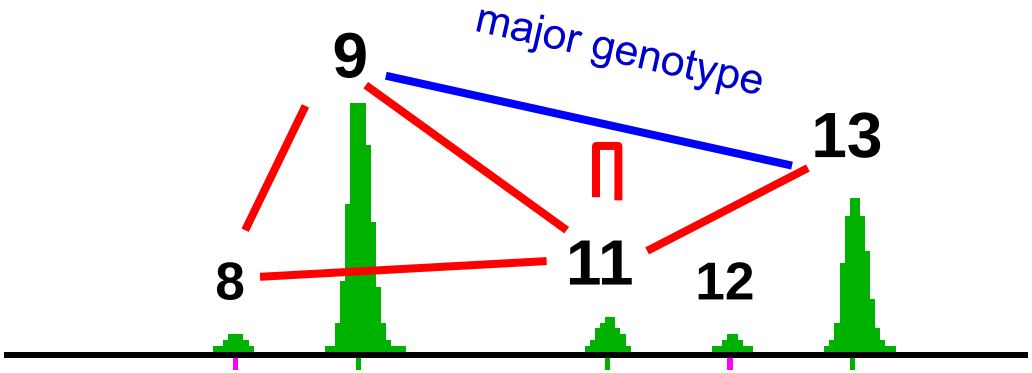
PHR, mix ratio, stutter, etc…

Typically thousands of simulations are performed

(MCMC)

Probable ***Genotypes*** to explain the mixture

D16S539



Minor Contributor Possible Genotypes	Probability
9,11	76%
11,11	15%
11,13	2%
8,11	2%
8,9	<1%
…	<1%

- Quantitative computer interpretation using numerous Markov Chain Monte Carlo (MCMC) simulations
- Models peak uncertainty and infers possible genotypes
- Results are presented as the Combined LR

Math Analogy to DNA Evidence

$2 + 2 = 4$

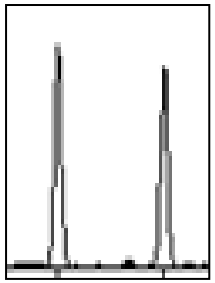
Basic Arithmetic

$2x^2 + x = 10$

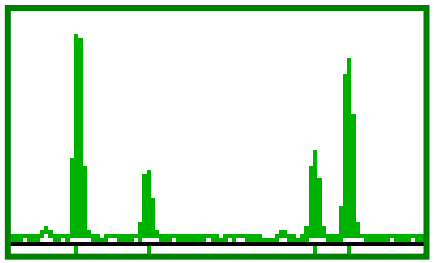
Algebra

$$\int_{x=0}^{\infty} f(x)\,dx$$

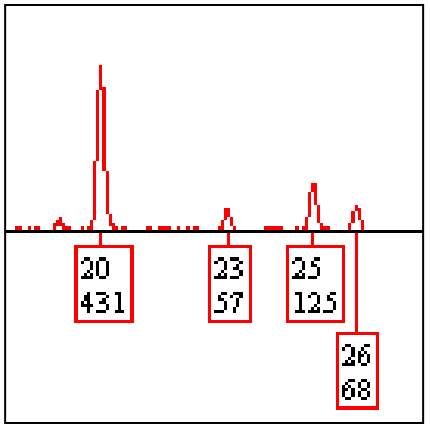
Calculus



Single-Source DNA Profile
(DNA databasing)



Sexual Assault Evidence
(2-person mixture with high-levels of DNA)



Touch Evidence
(>2-person, low-level, complex mixtures perhaps involving relatives)

Many laboratories are not prepared
to cope with complex mixtures

- Have **appropriate validation studies** been performed to inform proper interpretation protocols? (curriculum & classroom instruction)

- Are **appropriately challenging proficiency tests** being given? (graded homework assignments)

- **Would we want to go into a calculus exam only having studied algebra and having completed homework assignments involving basic arithmetic?**

Summary of DNA Mixture Interlaboratory Studies Conducted by NIST

Study	Year	# Labs	# Samples	Mixture Types
MSS 1	1997	22	11 stains	ss, 2p, 3p
MSS 2	1999	45	11 stains	ss, 2p, 3p
MSS 3	2000-01	74	7 extracts	ss, 2p, 3p
MIX05	2005	69	4 cases (.fsa)	only 2p
MIX13	2013	108	5 cases (.fsa)	2p, 3p, 4p

ss = single-source
2p = 2-person
3p = 3-person
4p = 4-person

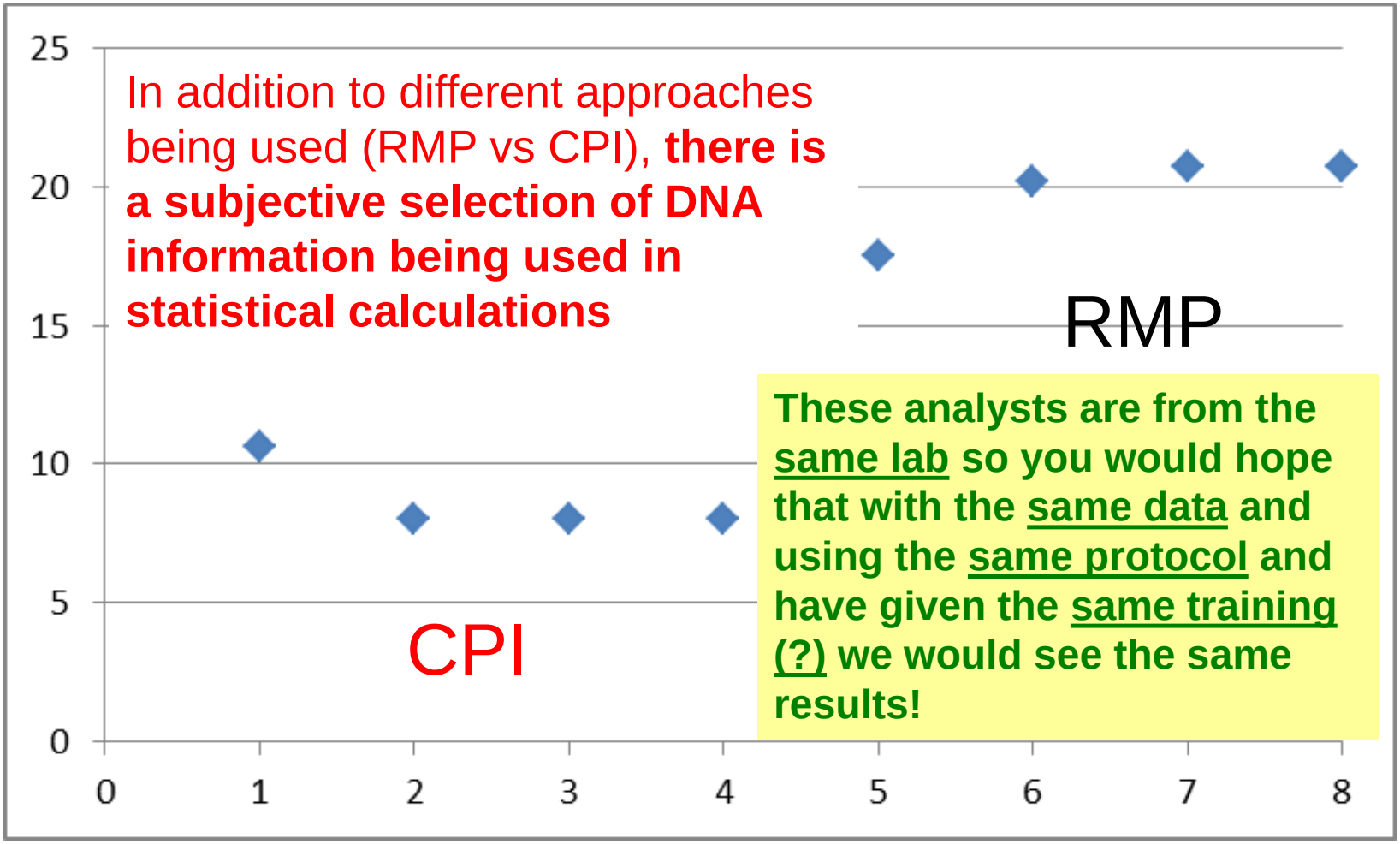
- Other recent studies
  - UK Regulator
  - USACIL

Studies have revealed significant variations in approaches among and within forensic laboratories

Slide from Mike Coble (NIST)

MIX13 Study Case 1 Results

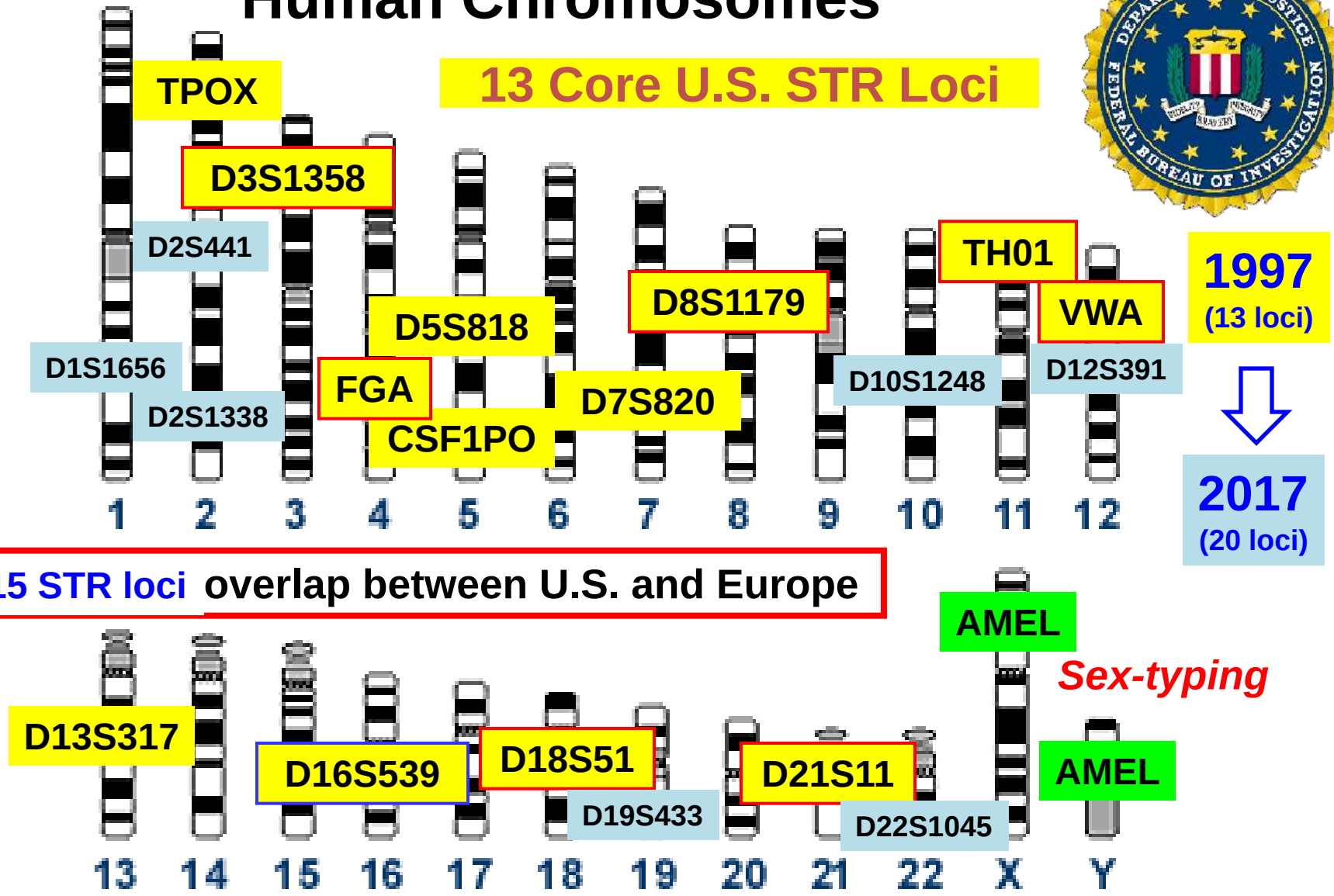
Intra-Laboratory Results (n = 8)



Data from Mike Coble (NIST)

Position of Forensic STR Markers on Human Chromosomes

Core STR Loci for the United States



Why are we where we are today?

- The incredible success of DNA has lead to **more sensitive methods and more "touch-evidence" samples being provided** which has led to more complex mixtures (we are pushing the envelope)
  - Lower template DNA profiles have more uncertainty associated with them in terms of allele peak height variation

- **Statistical interpretation techniques have not kept pace with the methodology improvements**
  - Much of the U.S. forensic DNA community is effectively using a 1992 statistical tool on 21st century data

Are We Facing a "Perfect Storm" for DNA Testing and Interpretation?

- Increase in assay and instrument sensitivity
- Increase in challenging casework samples (touch evidence)
- Increase in possible statistical tools for use with complex mixtures
- Increase in number of loci examined with new STR kits

http://allthingsd.com/files/2012/05/perfect-storm.jpeg

The Law Society Gazette

An article published Jan 12, 2015

DNA and case preparation
by David Bentley

Low-level complex mixtures

DNA mixtures introduce a whole new level of complexity, and a recent 'hot topic' has been how to interpret complex mixtures from low-level, incomplete samples [2]. Here, the conventional (and transparent) methods of analysis break down. Reporting analysts have been unable to provide any statistical basis for the possible inclusion of a match to a suspect's profile within such mixtures.

In a (controversial) decision [3], the Court of Appeal has permitted the limited use of subjective, non-statistically based opinions – where based on their 'experience', analysts will suggest that due to the number of matching alleles from the suspect's profile contained within the mixture, there is 'some' or 'moderate' support for the suspect being a contributor. This represents, some may think, a radical departure from the previous belief that DNA results had to be accompanied by a statistical weight, but as we will see later, this may prove to be no more than a temporary stopgap – as computerisation takes hold.

Practitioners may also have noticed that a new type of conclusion is appearing in DNA reports. It may be claimed that a mixed DNA sample recovered from a crime scene provides statistically based evidence against a suspect. If the report contains words and phrases such as 'low level', 'incomplete' or 'complex mixture', alarm bells should start to sound.

So too if, rather than giving a traditional RMP figure, the report sets up competing hypotheses (the prosecution hypothesis vs the defence hypothesis) and goes on to suggest that the former is 'x times more likely' than the latter. These are Likelihood Ratios (LRs), not RMPs, and require different analysis and understanding.

So if you come across any of the above, it is likely that you are now dealing with a wholly different set of challenges, arising from the use of a computerised model for interpretation.

If the report contains words and phrases such as 'low level', 'incomplete' or 'complex mixture', alarm bells should start to sound.

http://www.lawgazette.co.uk/law/practice-points/dna-and-case-preparation/5045883.fullarticle

Perhaps We Should Slow Down with Some of the DNA Mixtures That We (Scientists and Lawyers) Are Taking On…

Poor Quality Conditions

Large Numbers of Contributors

FORENSIC SCIENCE ERROR MANAGEMENT

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301-975-4049 john.butler@nist.gov