DNA Interpretation Workshop 2

Strengths and Limitations of Methods

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ISFG Pre-Conference Workshop
Melbourne, Australia
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NIST and NIJ Disclaimer

Funding: Interagency Agreement between the National Institute of Justice and NIST Office of Law Enforcement Standards

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.

Presentation Outline

- Observations and lessons from U.S. training courses conducted over the past several years
- Limitations of threshold-based approaches and CPI/RMNE statistics
- Probabilistic genotyping challenges

Final version of this presentation will be available at:
http://www.cstl.nist.gov/strbase/NISTpub.htm

Mixture Workshop Attendees

50 states and 25 other countries

Federal Labs
FBI
ATF
AFDIL
USACIL

DNA Mixture Interpretation

April 12, 2013 Webcast

NIST FORENSIC SCIENCES

http://www.nist.gov/oles/forensics/dna-analyst-training-on-mixture-interpretation.cfm

- **8-hours of DNA mixture interpretation training**
- **11 presentations** from **five different presenters**
  - John Butler, Mike Coble, Robin Cotton, Bruce Heidebrecht, Charlotte Word
- **20 poll questions** asked via SurveyMonkey (>600 participated)
  - Addressed additional questions sent via email or Twitter
- **>1000 participants** (almost entire U.S. represented and >10 countries)
- **Available for viewing or download** for at least six months (storage costs may limit longer-term storage)

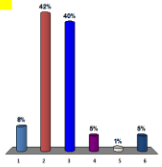
Left to right:
Gladys Arrisueno (NIST, Twitter feed monitor & poll questions)
John Paul Jones (NIST, webcast organizer)
Mike Coble (NIST, presenter)
John Butler (NIST, presenter & organizer)
Charlotte Word (Consultant, presenter)
Robin Cotton (Boston University, presenter)
Bruce Heidebrecht (Maryland State Police Lab, presenter)

Real-time interaction with the audience

How many DNA-related articles would you estimate that you read in a typical month?

Data from 2102 ISHI Mixture workshop (Oct 2012

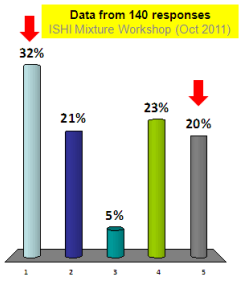
1. None
2. 1 article
3. 2 to 5 articles
4. More than 5 articles
5. None, I only read the abstracts
6. I don't make time to read!



2011 Response from ISHI Workshop

If your laboratory uses a stochastic threshold (ST), it is:

1. Same value as our analytical threshold (**we don't use a ST**)
2. About twice as high as our AT (e.g., AT = 50 and ST = 100 RFU)
3. Less than twice as high as our AT
4. Greater than twice as high as our AT
5. I don't know!



2012 Response from ISHI Workshop

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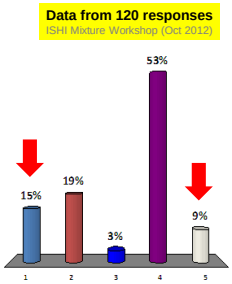


Coupling of Statistics and Interpretation

- **The CPE/CPI approach** for reporting an inclusionary statistic **requires that all alleles be observed** in the evidence sample
- If allele drop-out is suspected at a locus, then any allele is possible and the probability of inclusion goes to 100% -- in other words, the locus is effectively dropped from consideration
- If alleles are seen below the established stochastic threshold, then the locus is typically eliminated ("INC" – declared inconclusive) in many current lab SOPs

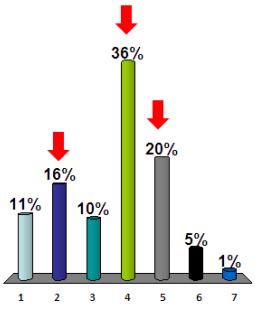
Use of CPI is still widespread in U.S.

What kind of mixture statistic does your lab use?

2011 Response at Training Workshop

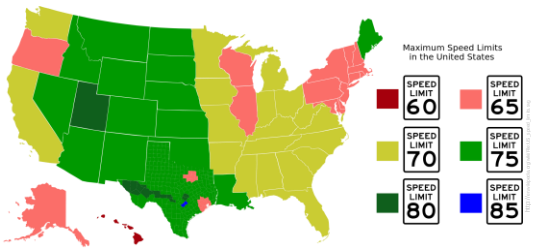
1. LR
2. CPE (RMNE, CPI)
3. RMP
4. CPE or RMP
5. Other combinations
6. Probabilistic modeling (e.g., TrueAllele)
7. We don't use stats (contradicting the new guidelines – section 4.1)

72% using CPI



Data from 138 responses
ISHI Mixture Workshop (Oct 2011)

A variety of approaches exist for how protocols and thresholds are set…



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

President John F. Kennedy

Yale University commencement address (June 11, 1962)

"For the greatest enemy of truth is very often not the lie – deliberate, contrived and dishonest – but the myth – persistent, persuasive, and unrealistic. Too often we hold fast to the clichés of our forebears. We subject all facts to a prefabricated set of interpretations. **We enjoy the comfort of opinion without the discomfort of thought**."

http://www.jfklibrary.org/Research/Ready-Reference/Kennedy-Library-Miscellaneous-Information/Yale-University-Commencement-Address.aspx

Written summary of a recent interview…

The CAC News • 1st Quarter 2012 pp. 8-11

norah rudin & keith inman • the proceedings of lunch

The Discomfort of Thought
—a discussion with John Butler

"For the greatest enemy of truth is very often not the lie – deliberate, contrived and dishonest – but the myth – persistent, persuasive, and unrealistic. Too often we hold fast to the clichés of our forebears. We subject all facts to a prefabricated set of interpretations. We enjoy the comfort of opinion without the discomfort of thought."
—John F. Kennedy

Available at http://www.cacnews.org/news/1stq12.pdf

"…**we should spend as much time developing our interpretation skills as we do our methodological skills.** Technological progress (more sensitivity in detecting DNA, for example), can be a double–edged sword; without equivalent progress in interpretation skill, we are just as likely to cut ourselves as we are the target."

"Your interpretation and statistical methods should have consistent assumptions and go together for each assumption being made (e.g., you may interpret a mixture under alternative sets of assumptions)…"

Results Depend on Assumptions

- "Although courts expect one simple answer, statisticians know that **the result depends on how questions are framed and on assumptions tucked into the analysis**."
  - Mark Buchanan, Conviction by numbers. *Nature* (18 Jan 2007) 445: 254-255

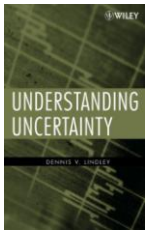
Uncertainty and Probability

- "Contrary to what many people think, **uncertainty is present throughout any scientific procedure**."
  - Dennis V. Lindley, in his foreword to Aitken & Taroni (2004) *Statistics and the Evaluation of Evidence for Forensic Scientists, Second Edition*
- "It is now recognized that **the only tool for handling uncertainty is probability**."
  - Dennis V. Lindley, in his foreword to Aitken & Taroni (2004) *Statistics and the Evaluation of Evidence for Forensic Scientists, Second Edition*

Whatever way uncertainty is approached, probability is the *only* sound way to think about it.

Understanding Uncertainty, p. 71

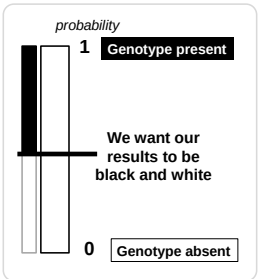
- Dennis Lindley



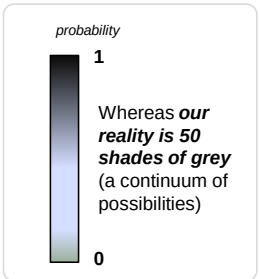
Wiley (2007)

Adapted from a slide by Peter Gill, Rome meeting, April 27-28, 2012: The hidden side of DNA profiles: artifacts, errors and uncertain evidence

Approaches to Data Interpretation: Binary vs Probabilistic



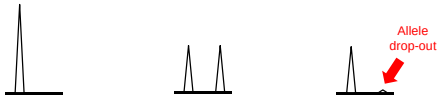
Binary Approach



Probabilistic Approach

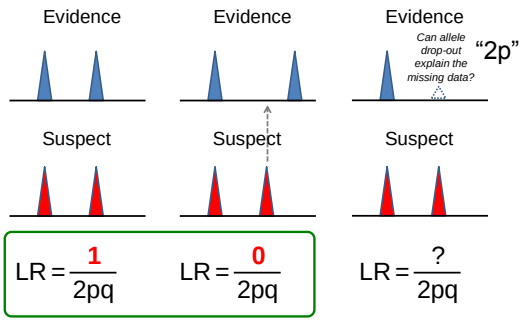
Allele Drop-out

- If because of chemistry events sometimes associated with low levels of DNA (termed "stochastic effects"), one of the STR alleles "drop-out" and is not detected, then our sample at that locus looks like a homozygote instead of the heterozygote that it really is



True homozygote (only a single peak)	**True heterozygote** (both peaks detected)	**False homozygote** (one peak has "dropped out" and fails to be detected)
Statistical treatment: p^2	$2pq$	$2p$

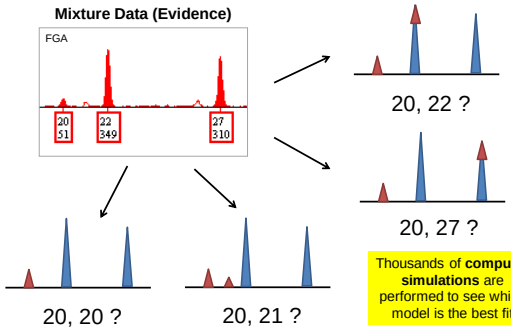
Likelihood Ratios for Different Possibilities



Binary LR approach (either 0 or 1)

Modified slide from Mike Coble (NIST)

Probabilistic Genotyping Involves Exploring Multiple Possibilities to See Which One Best Fits the Data

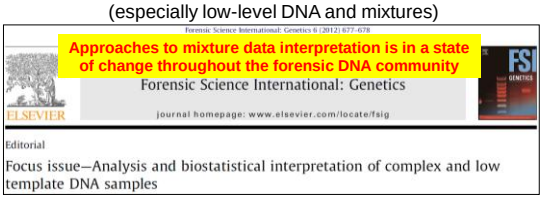


Thousands of **computer simulations** are performed to see which model is the best fit

Slide adapted from Mike Coble (NIST)

New Efforts to Improve DNA Interpretation

(especially low-level DNA and mixtures)



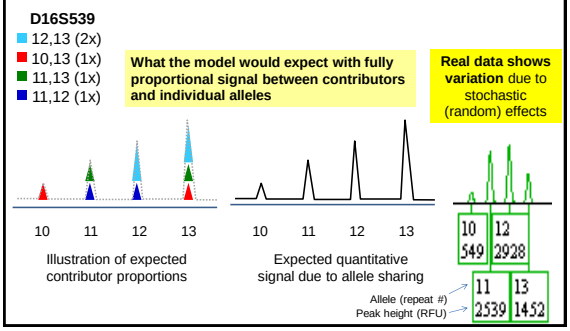
December 2012 – Forensic Science International: Genetics, volume 6, issue 6

STRmix approach has recently been published

New Statistical Tools/Software for Mixtures

- **Lab Retriever** (David Balding → Norah Rudin et al.)
  - Uses likelihood ratios (LRs) and probability of dropout [Pr(D) or P(Do)]
- **FST** – Forensic Statistical Tool (NYC OCME)
  - Uses LRs and empirically determined Pr(D) based on DNA quantity
- **Armed Xpert** (USACIL → Niche Vision)
  - Originally developed by US Army Crime Lab (USACIL)
  - Performs calculations typically manually done by analysts
- **TrueAllele** (Mark Perlin/Cybergenetics)
  - Uses probabilistic genotyping approach with LRs
- **STRmix** (John Buckleton/New Zealand ESR)
  - Like TrueAllele, uses LRs with computer simulations

Impact of Allele Sharing on Ability to Clearly Determine the Number of Contributors



Handling Complex Mixtures

- Stochastic thresholds are necessary in combination with CPI statistics
  - but a stochastic threshold may not hold much meaning for >2 person mixtures (due to potential allele sharing)
- Most labs are not adequately equipped to cope with complex mixtures
  - Extrapolating validation studies from simple mixtures will not be enough to create appropriate interpretation SOPs

David Balding (UK professor of statistical genetics): "LTDNA cases are coming to court **with limited abilities for sound interpretation**." (Rome, April 2012 meeting)

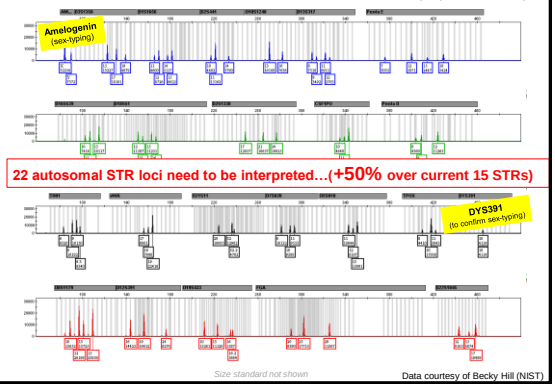
Thoughts on Where We Need to Go (1)

- Away from CPI and towards likelihood ratio approaches
  - As noted in the Gill et al. (2006) ISFG DNA Commission recommendation #2
- This will require software to perform the calculations
  - This software will need to be validated
  - Peter Gill and others are pushing freeware solutions
- Still will require analysts to understand what is going on in the computer calculations!
  - Will require more significant engagement in mixture training

Thoughts on Where We Need to Go (2)

- Validation studies need to support interpretation SOPs and software packages
- The U.S. will be moving to more STR loci in the near future (from 13 to ~20 core STRs)
  - Using additional loci with better powers of discrimination will improve detection of mixtures
  - **But more loci means more interpretation time!**

DNA Mixture Detected with PowerPlex Fusion (24plex STR kit)



Data courtesy of Becky Hill (NIST)

Where Can Potential Errors Occur in DNA Interpretation?

- Incorrect inclusion of an innocent person using allele drop-out as a reason for mismatch between evidence and suspect with a CPI approach
- Inclusion of loci in CPI calculations with alleles below stochastic threshold (CPI requires all alleles to be detected)
  - Could lead to an inflation of match statistic
- Setting thresholds too high
  - Loss of relevant data that could be used to exclude
- Use of p^2 instead of 2p with single peaks where allele dropout may have happened
  - Will falsely inflate stats
- Failure to exclude when alleles are present but genotypes do not fit

Is the Known Individual Included or Excluded?

Known: 13,14

Known: 28,30

Assumptions:
1) 2 contributors *and* all data are present →
2) 1 major and 1 minor contributor →
3) Major must have 13,16 and 28,28 genotypes and
4) Minor must have 14,15 and 30,32.2 genotypes

Based on these assumptions, the individual is excluded

Genotypes are excluded even if alleles are included

Slide from Charlotte Word (consultant)

Different Experts→ Different Opinions

- Are the experts asking/answering the same question?
- Are they using the same information and data?
- Are they using the same interpretation methods?
- Are they using good scientific practices?
- Any possibility of bias?
- Are the differences meaningful or trivial?

Slide from Charlotte Word (consultant)

Greg Matheson on Forensic Science Philosophy

The CAC News – 2nd Quarter 2012 – p. 6
"Generalist vs. Specialist: a Philosophical Approach"
http://www.cacnews.org/news/2ndq12.pdf

- If you want to be a technician, performing tests on requests, then just focus on the policies and procedures of your laboratory. If you want to be a scientist and a professional, learn the policies and procedures, but go much further and learn the philosophy of your profession. **Understand the importance of why things are done** the way they are done, the scientific method, the viewpoint of the critiques, the issues of bias and the importance of ethics.

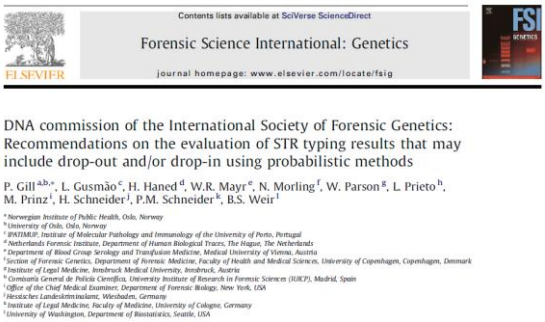
Some Thoughts on the Future…

- **PCR amplification**
  - Faster enzymes to enable rapid PCR
  - More robust enzymes and master mixes to overcome inhibition
- **Instrumentation**
  - More dye colors to aid higher levels of multiplexing
  - Rapid, integrated devices
  - Alternatives to capillary electrophoresis: next-generation sequencing
- **Marker systems**
  - Expanding sets of STR loci for growing DNA databases
  - Other marker systems: SNPs, InDels, X-STRs, RM Y-STRs
  - Body fluid identification with mRNA, miRNA, and DNA methylation
  - Phenotyping for external visible characteristics
  - Privacy challenges with additional genome information
- **Data interpretation**
  - Probabilistic genotyping for low-level DNA and mixture interpretation
  - Probability of dropout incorporated into DNA data interpretation

Summary of the Issues

- New kits, new instruments will only increase the difficulties of interpreting low-level, challenging samples.
- If we are really serious about properly interpreting low level and complex mixtures, we must move away from the threshold-based CPI/RMNE mentality.
- Probabilistic methods are the way forward and a number of software programs are available ranging from "open source" to commercial packages.

December 2012 Issue of *FSI Genetics* is on **DNA Interpretation Challenges and Solutions**


Contents lists available at SciVerse ScienceDirect

Forensic Science International: Genetics

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

DNA commission of the International Society of Forensic Genetics: Recommendations on the evaluation of STR typing results that may include drop-out and/or drop-in using probabilistic methods

P. Gill[a,b,*], L. Gusmão[c], H. Haned[d], W.R. Mayr[e], N. Morling[f], W. Parson[g], L. Prieto[h], M. Prinz[i], H. Schneider[j], P.M. Schneider[k], B.S. Weir[l]

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[d] Netherlands Forensic Institute, Department of Human Biological Traces, The Hague, The Netherlands
[e] Department of Blood Group Serology and Transfusion Medicine, Medical University of Vienna, Austria
[f] Section of Forensic Genetics, Department of Forensic Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
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[h] Comisaría General de Policía Científica, University Institute of Research in Forensic Sciences (IUICP), Madrid, Spain
[i] Office of the Chief Medical Examiner, Department of Forensic Biology, New York, USA
[j] Hessisches Landeskriminalamt, Wiesbaden, Germany
[k] Institute of Legal Medicine, Faculty of Medicine, University of Cologne, Germany
[l] University of Washington, Department of Biostatistics, Seattle, USA

Challenge of Transitioning between Methods

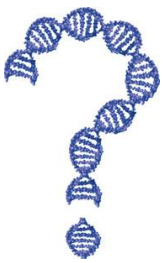
- Most labs are not allowed to shut down in order to have time to learn new procedures.
- Analysts have to learn new procedures while issuing reports under current policies.
- This transition period can be very frustrating.

Slide from Bruce Heidebrecht (NIST webcast, 12 April 2013)

Acknowledgments

$ National Institute of Justice and NIST OLES
John Buckleton and co-presenters

Slides and Discussions on DNA Mixtures
Mike Coble (NIST Applied Genetics Group)
Robin Cotton & Catherine Grgicak (Boston U.)
Bruce Heidebrecht (Maryland State Police)
Charlotte Word (consultant)



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Final version of this presentation will be available at:
http://www.cstl.nist.gov/strbase/NISTpub.htm