Forensic DNA Mixture Interpretation

Statistical Approaches for DNA Mixtures

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Why Do Stats?

- A way to assess the weight of the statement that two profiles match (or cannot be excluded as originating from the same source)
- To be non-prejudicial

Stats Required for Inclusions

SWGDAM Interpretation Guideline 4.1:

"The laboratory must perform statistical analysis in support of any inclusion that is determined to be relevant in the context of a case, irrespective of the number of alleles detected and the quantitative value of the statistical analysis."

Buckleton & Curran (2008): "There is a considerable aura to DNA evidence. Because of this aura it is vital that weak evidence is correctly represented as weak or not presented at all."

Buckleton, J. and Curran, J. (2008) A discussion of the merits of random man not excluded and likelihood ratios. *Forensic Sci. Int. Genet.* 2: 343-348.

See Ladd et al. (2001) Croat Med J. 42:244-246

"Exclusionary" Approach

Random Man Not Excluded (RMNE)

Combined Prob. of Inclusion (CPI)

Combined Prob. of Exclusion (CPE)

"Allele-centric"

"Inferred Genotype" Approach

Random Match Probability [modified] (mRMP)

> Likelihood Ratio (LR)

"Genotype-centric"

 Random Man Not Excluded (CPE/CPI) - The probability that a random person (unrelated individual) would be excluded as a contributor to the observed DNA mixture.



$$PI = (f(a) + f(b) + f(c) + f(d))^2$$

 $CPI = PI_{M1} X PI_{M2} \cdots$
 $CPE = 1 - CP1$

modified Random Match Probability (mRMP)

 The major and minor components can be successfully separated into individual profiles. A random match probability is calculated on the evidence as if the component was from a single source sample.



 Likelihood Ratio - Comparing the probability of observing the mixture data under two (or more) alternative hypotheses

Probability

- Can be either...
- The frequency of observing an event in a large number of trials (Frequentist)
- The subjective degree of belief (Bayesian)

Probability

- Frequentist What is the coincidental chance of the observed event.
- Bayesian determine a <u>posterior probability</u> of observing the event based upon the data + the <u>prior probability</u> based upon knowledge of the source.

Laws of Probability

- Probabilities can range from 0 to 1.
- Events can be mutually exclusive (add)

P(G or H|E) = P(G|E) + P(H|E)

• Events can be independent (multiply)

$P(G \text{ and } H|E) = P(G|E) \times P(H|E)$

Probabilities

- What is the probability of rolling a "5" using a sixsided die?
- P(rolling a 5) = 1/6
- What is the probability of rolling a "5" or "6"?
- P(rolling a 5) + P(rolling a 6) = 1/6+1/6 = 2/6 or 1/3.

Probabilities

- What is the probability of rolling a "5" on the first throw and rolling a "6" on the second roll?
- P(rolling a 5) * P(rolling a 6) = 1/6*1/6 = 1/36.

$$1,1$$
 $2,1$ $3,1$ $4,1$ $5,1$ $6,1$ $1,2$ $2,2$ $3,2$ $4,2$ $5,2$ $6,2$ $1,3$ $2,3$ $3,3$ $4,3$ $5,3$ $6,3$ $1,4$ $2,4$ $3,4$ $4,4$ $5,4$ $6,4$ $1,5$ $2,5$ $3,5$ $4,5$ $5,5$ $6,5$ $1,6$ $2,6$ $3,6$ $4,6$ $5,6$ $6,6$

Conditioning

- **Probabilities are conditional**, which means that the probability of something is based on a hypothesis
- In math terms, conditioning is denoted by a vertical bar
 Hence, Pr(a|b) means 'the probability of a <u>given</u> that b is true"
- The probability of an event *a* is dependent upon various assumptions—and these assumptions or hypotheses can change...

Probability Example – Will It Rain? (1)

Defining the Event and Assumptions/Hypotheses

- Let's suppose that *a* is the probability of an event (e.g., will it rain?)
- What is the probability that it will rain in the afternoon Pr(a)?
- This probability is dependent upon assumptions
 - We can look at the window in the morning and observe if it is sunny (s) or cloudy (c)
 - Pr(a) **<u>if</u>** it is sunny (s) is less than Pr(a) **<u>if</u>** it is cloudy (c)
- We can write this as Pr(a/s) and Pr(a/c)
 - Since sunny or cloudy are the only possibilities, Pr(s) + Pr(c) = 1
 - or Pr(s) = 1 Pr(c)

Probability Example – Will It Rain? (2)

Examining Available Data

- Pr(a|s) and Pr(a|c) can be calculated from data
- How often does it rain in the afternoon when its sunny in the morning?
 - 10 out of 100 observations so Pr(a|s) = 0.1
- How often does it rain in the afternoon when it is cloudy in the morning?
 - 90 out of 100 observations so Pr(a|c) = 0.9

Probability Example – Will It Rain? (3)

Formation of the Likelihood Ratio (LR)

 The LR compares two probabilities to find out which of the two probabilities is the most likely

The probability that it will rain in the afternoon when it is cloudy in the morning or Pr(a|c) is divided by the probability that it will rain in the afternoon when it is sunny in the morning or Pr(a|s)

$$LR = \frac{\Pr(a \mid c)}{\Pr(a \mid s)} = \frac{0.9}{0.1} = 9$$

Slide information from Peter Gill (ISFG 2007 workshop, Copenhagen, August 20-21, 2007)

Probability Example – Will It Rain? (4)

Explanation of the Likelihood Ratio

$$LR = \frac{\Pr(a \mid c)}{\Pr(a \mid s)} = \frac{0.9}{0.1} = 9$$

- The probability that it will rain is 9 times more likely <u>if</u> it is cloudy in the morning than <u>if</u> it is sunny in the morning.
- The word **<u>if</u>** is very important here. It must always be used when explaining a likelihood ratio otherwise the explanation could be misleading.

Likelihood Ratios in Forensic DNA Work

- We evaluate the evidence (*E*) relative to alternative pairs of hypotheses
- Usually these hypotheses are formulated as follows:
 - The probability of the evidence if the crime stain originated with the suspect or Pr(*E*/*S*)
 - The probability of the evidence if the crime stain originated from an unknown, unrelated individual or Pr(E|U)

$$LR = \frac{\Pr(E \mid S)}{\Pr(E \mid U)} \longleftarrow \text{The numerator}$$

The Likelihood Ratio Must Be Stated Carefully

- The probability of the evidence is *x* times more likely if the stain came from the suspect Mr. Smith than if it came from an unknown, unrelated individual.
- It is not appropriate to say: "The probability that the stain came from Mr. Smith." because we must always include the conditioning statement – i.e., always make the hypothesis clear in the statement.
- Always use the word 'if' when using a likelihood ratio to avoid this trap

Likelihood Ratio (LR)

 Provides ability to express and evaluate both the prosecution hypothesis, H_p (the suspect is the perpetrator) and the defense hypothesis, H_d (an unknown individual with a matching profile is the perpetrator)

$$LR = \frac{H_p}{H_d}$$

- The numerator, H_p, is usually 1 since in theory the prosecution would only prosecute the suspect if they are 100% certain he/she is the perpetrator
- The denominator, H_d, is typically the profile frequency in a particular population (based on individual allele frequencies and assuming HWE) i.e., the random match probability

 Likelihood Ratio - Comparing the probability of observing the mixture data under two (or more) alternative hypotheses; in its simplest form LR = 1/RMP



Last Year's Response What kind of mixture statistic does your lab use? 72% using CPI 1. LR

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

Data from 138 responses ISHI Mixture Workshop (Oct 2011)





Forensic Science International: Genetics 2 (2008) 343-348

A discussion of the merits of random man not excluded and likelihood ratios

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We conclude that the two matters that appear to have real force are:

(1) LRs are more difficult to present in court and(2) the RMNE statistic wastes information that should be utilised.

CPE/CPI (RMNE) Limitations

- A CPE/CPI approach assumes that all alleles are present (i.e., cannot handle allele drop-out)
- Thus, statistical analysis of low-level DNA CANNOT be correctly performed with a CPE/CPI approach because some alleles may be missing
- Charles Brenner in his AAFS 2011 talk addressed this issue
- Research is on-going to develop allele drop-out models and software to enable appropriate calculations

Notes from Charles Brenner's AAFS 2011 talk

The Mythical "Exclusion" Method for Analyzing DNA Mixtures – Does it Make Any Sense at All?

- 1. The claim that it requires **no assumption about number of contributors** is mostly wrong.
- 2. The supposed **ease of understanding** by judge or jury is really an illusion.
- 3. Ease of use is claimed to be an advantage particularly for complicated mixture profiles, those with many peaks of varying heights. The truth is the exact opposite. The exclusion method is completely invalid for complicated mixtures.
- 4. The exclusion method is only **conservative** for guilty suspects.
- **Conclusion:** "Certainly no one has laid out an explicit and rigorous chain of reasoning from first principles to support the exclusion method. It is at best guesswork."

Brenner, C.H. (2011). The mythical "exclusion" method for analyzing DNA mixtures – does it make any sense at all? *Proceedings of the American Academy of Forensic Sciences*, Feb 2011, Volume 17, p. 79

DAB Recommendations on Statistics

February 23, 2000 Forensic Sci. Comm. 2(3); available on-line at http://www.fbi.gov/hq/lab/fsc/backissu/july2000/dnastat.htm

"The DAB finds either one or both PE or LR calculations acceptable and strongly recommends that one or both calculations be carried out whenever feasible and a mixture is indicated"

- Probability of exclusion (PE)
 - Devlin, B. (1993) Forensic inference from genetic markers. Statistical Methods in Medical Research 2: 241–262.
- Likelihood ratios (LR)
 - Evett, I. W. and Weir, B. S. (1998) *Interpreting DNA Evidence*. Sinauer, Sunderland, Massachusetts.

Statistical Methods in Medical Research 1993; 2: 241-262

Forensic inference from genetic markers

B Devlin Department of Epidemiology and Public Health, Yale University School of Medicine

Section 5.1 Exclusion probability

- Discussion about exclusion probabilities in Paternity cases.

Two types:

(1) Conditional Exclusion Probability - excluding a random man as a possible father, given the mother-child genotypes for a particular case.

(2) Average Exclusion Probability – excluding a random man as a possible father, given a randomly chosen mother-child pair.

Forensic inference from genetic markers

B Devlin Department of Epidemiology and Public Health, Yale University School of Medicine

Section 5.1 Exclusion probability

"The theoretical concept of exclusion probabilities, however, makes no sense within the framework of normal mixture models."

"The interpretation of conditional exclusion probability is obvious, which accounts for its value in the legal arena. Unlike [LR], however, it is not fully efficient."





Curran and Buckleton (2010)





J Forensic Sci, September 2010, Vol. 55, No. 5 doi: 10.1111/j.1556-4029.2010.01446.x Available online at: interscience.wiley.com

PAPER CRIMINALISTICS; GENERAL

James M. Curran,¹ M.Sc.(Hons.), Ph.D. and John Buckleton,² Ph.D.

Inclusion Probabilities and Dropout

Created 1000 Two-person Mixtures (Budowle et al. 1999 AfAm freq.).

Created 10,000 "third person" genotypes.

Compared "third person" to mixture data, calculated PI for included loci, ignored discordant alleles.

Curran and Buckleton (2010)



Impact of Dropping Loci

- The less data available for comparison purposes, the greater the chance of falsely including someone who is truly innocent
- Are you then being "conservative" (i.e., erring in favor of the defendant)?

If CPI/CPE Stats are Used

Since exclusionary statistics cannot adjust for the possibility of dropout, and does not take the number of contributors into account, any loci where alleles are below stochastic levels cannot be used in the CPI statistic.

If CPI/CPE Stats are Used





0

If CPI/CPE Stats are Used

Can use D21 CSF **D**3 **D19** TPOX

<u>Cannot use</u>	
D8	D2
D7	vWA
TH01	D18
D13	D5
D16	FGA
If CPI/CPE Stats are Used

- CPI statistics using FBI Caucasian Frequencies
- 1 in 71 Caucasians included
- 98.59% Caucasians excluded

If RMP/LR Stats are Used

• Since there is an assumption to the number of contributors, it is possible to use data that falls below the ST.

RMP - D18S51



(LR = 113)

If Assume 2 Contributors		
<u>Major</u>	<u>Minor</u>	
16,18	14,20	

 $RMP_{minor} = 2pq$ = 2 x f(14) x f(20) = 2 x (0.1735) x (0.0255) = 0.00884 or 1 in 113





RMP = 8,11 + 11,11RMP = $2pq + (q^2 + q(1-q)\theta)$

 $RMP = 2(0.5443)(0.2537) + (0.2537)^2 + (0.2537)(0.7463)(0.01) = 0.3424 \text{ or } 1 \text{ in } 2.9$



Profile 1: ID_2_SCD_NG0.5_R4,1_A1_V1.2



If RMP/LR Stats are Used

<u>Can use</u>	Loci with potential D-out	
D8	D7	D2
D21	TH01	ν/Λ/ Δ
D18		
D3	D13	D5
D19	D16	
TPOX		
FGA		
CSF		

The "2p" Rule

 The "2p" rule can be used to statistically account for zygosity ambiguity – i.e. is this single peak below the stochastic threshold the result of a homozygous genotype or the result of a heterozygous genotype with allele drop-out of the sister allele?





Resolved Question

Show me another »

To pee or not to pee? That is the question ...?

"Drink sir, is a great provoker of three things.... nose painting, sleep and urine."

Macbeth: Act 2, Scene 3

2p – SWGDAM Guidelines

- 5.2.1.3.1. The formula 2p, as described in recommendation 4.1 of NRCII, may be applied to this result.
- 5.2.1.3.2. Instead of using 2p, the algebraically identical formulae 2p p² and p² + 2p(1-p) may be used to address this situation without double-counting the proportion of homozygotes in the population.

2p – **p**² and **p**² + **2p(1-p)**

Suppose 5 allele system – P, Q, R, S & T



Profile 1 - TH01



Major – 7	7	
Possible Minor Contributors		
7, 9.3	(2pq)	
9.3, 9.3	p ²	
9.3, ?	2p (or p ² + 2p(1 – p))	

Profile 1 - TH01 (LR)



Profile 1 - TH01 (LR)

 $\frac{P(E|H_1)}{P(E|H_2)} = \frac{V \& S}{V \& U} = \frac{1}{p^2 + p(1-p)\theta + 2pq}$

V = 7, 7 U = 7, 9.3 9.3, 9.3 $= \frac{1}{f_{9.3}^2 + f_{9.3} (1 - f_{9.3})\theta + 2f_{9.3}f_7}$

Let ST = 125 RFU $f_{9.3} = 0.3054 = 1 / 0.2007 = 4.98$ $f_{7} = 0.1724$

The "2p" Rule

• "This rule arose during the VNTR era. At that time many smaller alleles "ran off the end of the gel" and were not visualised."

- Buckleton and Triggs (2006)

"Is the 2*p* rule always conservative?"



The "2p" Rule



Gill and Buckleton (2010)



Challenges with low level, complex mixtures





Clayton et al. (1998) ISFG (2006) Rec. #4



Impact of Results with Low Level DNA

When amplifying low amounts of DNA (e.g., 125 pg), allele dropout is a likely possibility leading to **higher uncertainty** in the potential number of contributors and in the possible genotype combinations





What Can We Say about this Result?

- Low level DNA (only amplified 125 pg total DNA)
 - likely to exhibit stochastic effects and have allele dropout
- Mixture of at least 3 contributors
 - Based on detection of 5 alleles at D18S51
 - If at equal amounts, ~40 pg of each contributor (if not equal, then less for the minor contributors); we expect allele dropout
- At least one of the contributors is male
 - Based on presence of Y allele at amelogenin
- Statistics if using CPI/CPE
 - Would appear that we can only use TPOX and D5S818 results with a stochastic threshold of 150 RFU (will explore this further)
- Due to potential of excessive allele dropout, we are unable to perform any meaningful Q-K comparisons

Uncertainty in the Potential Number of Contributors with this Result



5 alleles observed

Several of the peaks are barely above the analytical threshold of 30 RFU

In fact, with an analytical threshold of 50 RFU or even 35 RFU, there would only be three detected alleles at D18S51

- Stochastic effects could result in a high degree of stutter off of the 17 allele making alleles 16 and 18 potential stutter products
- No other loci have >4 alleles detected

All Detected Alleles Are Above the Stochastic Threshold – Or Are They?



Stochastic threshold = 150 RFU Does this result guarantee no allele drop-out?

We have assumed three contributors. If result is from an equal contribution of 3 individuals...

Then some alleles from individual contributors would be below the stochastic threshold and we could not assume that all alleles are being observed!



All Loci Are Not Created Equal when it comes to mixture interpretation

- In the case of less polymorphic loci, such as TPOX, there are fewer alleles and these occur at higher frequency. Thus, there is a greater chance of allele sharing (peak height stacking) in mixtures.
- Higher locus heterozygosity is advantageous for mixture interpretation – we would expect to see more alleles (within and between contributors) and thus have a better chance of estimating the true number of contributors to the mixture

Even if you did attempt to calculate a CPI/CPE statistic using loci with all observed alleles above the stochastic threshold on this result...



Impact of Amplifying More DNA



125 pg total DNA amplified 500 pg total DNA amplified

How should you handle the suspect comparison(s) with this case result?

- No suspect comparisons should be made as the mixture result has too much uncertainty with stochastic effects that may not account for all alleles being detected
- Declare the result "inconclusive"

How not to handle this result

- "To heck with the analytical and stochastic thresholds", I am just going to see if the suspect profile(s) can fit into the mixture allele pattern observed – and then if an allele is not present in the evidentiary sample try to explain it with possible allele dropout due to stochastic effects
- This is what Bill Thompson calls "painting the target around the arrow (matching profile)..."

Thompson, W.C. (2009) Painting the target around the matching profile: the Texas sharpshooter fallacy in forensic DNA interpretation. *Law, Probability and Risk* 8: 257-276

What to do with low level DNA mixtures?

- German Stain Commission "Category C" (Schneider et al. 2006, 2009)
 - Cannot perform stats because stochastic effects make it uncertain that all alleles are accounted for
- **ISFG Recommendations #8 & #9** (Gill et al. 2006)
 - Stochastic effects limit usefulness
- Fundamentals of Forensic DNA Typing (2010)
 Butler 3rd edition (volume 1), chapter 18
 Dep't ge "outside the bay" without supporting validation
 - Don't go "outside the box" without supporting validation



ISFG Recommendations on Mixture Interpretation

http://www.isfg.org/Publication;Gill2006

- 1. The likelihood ratio (LR) is the preferred statistical method for mixtures over RMNE
- 2. Scientists should be trained in and use LRs
- 3. Methods to calculate LRs of mixtures are cited
- 4. Follow Clayton et al. (1998) guidelines when deducing component genotypes
- 5. Prosecution determines H_p and defense determines H_d and multiple propositions may be evaluated

- 6. When minor alleles are the same size as stutters of major alleles, then they are indistinguishable
- Allele dropout to explain evidence can only be used with low signal data
- 8. No statistical interpretation should be performed on alleles below threshold
- 9. Stochastic effects limit usefulness of heterozygote balance and mixture proportion estimates with low level DNA

Gill *et al.* (2006) DNA Commission of the International Society of Forensic Genetics: Recommendations on the interpretation of mixtures. *Forensic Sci. Int.* 160: 90-101



A Complexity/Uncertainty Threshold

New Scientist article (August 2010)

- How DNA evidence creates victims of chance
 - 18 August 2010 by Linda Geddes
- From the last paragraph:
 - In really complex cases, analysts need to be able to draw a line and say "This is just too complex, I can't make the call on it," says Butler. "Part of the challenge now, is that every lab has that line set at a different place. But the honest thing to do as a scientist is to say: I'm not going to try to get something that won't be reliable."

Has your laboratory implemented a "stop testing" approach with complex and/or low-level mixture?

- 1. Yes
- 2. No
- 3. I don't work in a lab



Is there a way forward?

Thank You!

Our team publications and presentations are available at: http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm

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Questions?

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