



DNA Mixture Interpretation Workshop | *Jack Ballantyne*

***Evolution of DNA Mixture Interpretation***  
***(DNA Advisory Board and pre-2010 SWGDAM Guidelines)***



**DONT DROP OUT!**



**2011  
EDITION**

# **HISTORY OF DNA MIXTURES FOR NEWBIES**

**WITHOUT THE  
NEED FOR A  
PHD IN  
STATISTICS  
(BUT IT DOES  
HELP!)**

**IT'S NOT EASY  
WHEN YOU KNOW  
HOW**



[www.txt2pic.com](http://www.txt2pic.com)

1. Historical preamble showcasing ancient technology
2. Science of mixture analysis
3. Community consensus efforts

***“Those who cannot learn from  
history are doomed to repeat it”***

**George Santayana**  
***The Life of Reason (1905-1906)***

# ***Tasteful Statistics Jokes***

- **A statistician is a professional who diligently collects facts and data and then carefully draws confusions about them**
- **You can lie with statistics but even better without**
- **Statistics means you never have to say you're certain (wrong)**

# ***Seriously True Precepts***

- **All models are wrong. Some are useful.**
  - **George Box**
- **There are no facts, only interpretations.**
  - **Frederick Nietzsche**

# ***“Mixtures for Newbies”***

- 1. Recognize Mixture**
- 2. Infer Genotype(s)**
- 3. Attach Weight with Stats**

# Pre-DNA Era



National Library of Medicine

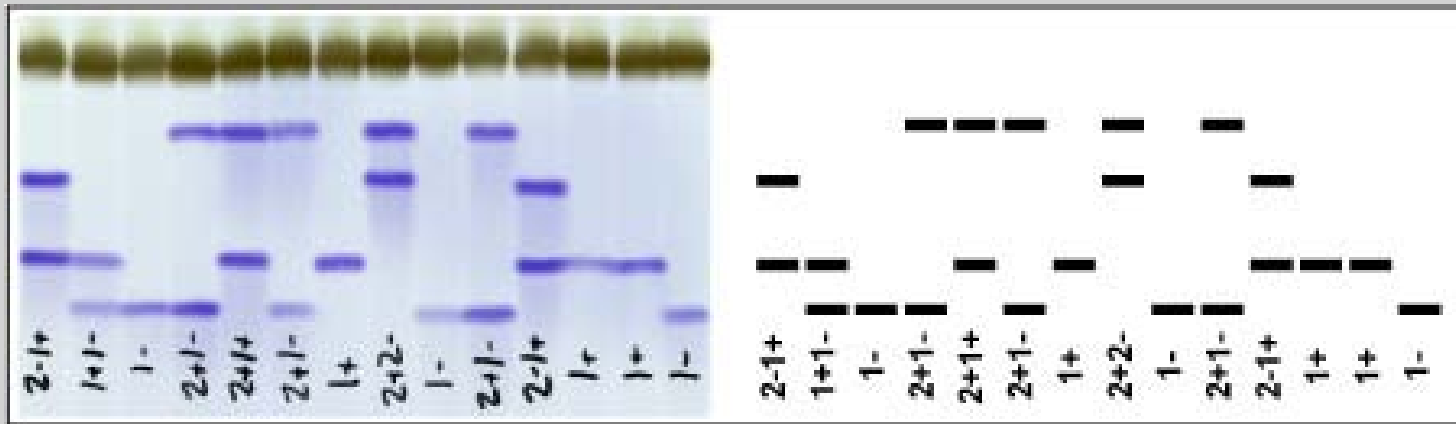
# ***Conventional Markers***

## ***ABO***

- **Victim is ‘B secretor’**
- **Vaginal swab with semen: ‘AB’**
- **Conclusion: mixture present with the semen donor being an A or AB**
- **Thus  $27\% + 6\% = 33\%$  of the Hong Kong Chinese male population cannot be excluded as donors of the semen stain**



# Enzymes: PGM



## For a single genetic marker system

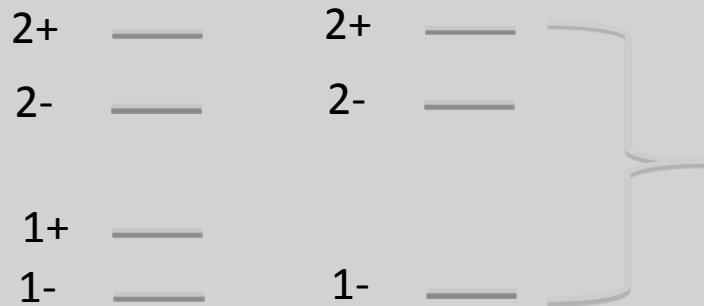
To **recognize** a mixture (no assumption of the presence of a particular donor) need genetic marker with  $\geq 3$  alleles (e.g. EAP, Gc, PGM)

To **eliminate** a proportion of the population as potential contributors need  $\geq 4$  alleles (only 1 system..PGM)

## For a multi-locus genetic marker system

To **recognize** a mixture (no assumption of the presence of a particular donor) need at least one genetic marker with  $\geq 3$  alleles

To **eliminate** a proportion of the population as potential contributors need loci with at least one common allele missing from the mixture



PGM Genotypes INCLUDED:

2+2+, 2-2-, 1-1-, 2+2-, 2+1-, 2-1-

= 3.1 + 0.8 + 1.8 + 3.1 + 4.6 + 2.8

= 16.2

Thus 16.2% of the population cannot be excluded (i.e. included) as potential doors of the stain

PGM Genotype	Observed Frequency	Allele Frequency
1+	35.7	0.60
1-	1.8	0.13
1+1-	15.9	
2+	3.0	0.17
2-	0.8	0.10
2+2-	3.1	
2+1+	19.7	
2+1-	4.6	
2-1+	11.8	
2-1-	2.8	

**However, 2+2+, 2-2-, 1-1-, 2+2-, 2+1-, 2-1-**  
**=  $p_{2+}^2 + p_{2-}^2 + p_{1-}^2 + 2p_{2+}p_{2-} + 2p_{2+}p_{1-} + 2p_{2-}p_{1-}$**

[Where allele frequencies are  $p_{2+}$ ,  $p_{2-}$ ,  $p_{1+}$ ,  $p_{1-}$   
and  $(p_{2+} + p_{2-} + p_{1+} + p_{1-}) = 1$  ]

**=  $(p_{2+} + p_{2-} + p_{1-})^2 = \text{Probability of Inclusion} = \text{PI}$**   
**= RMNE = 0.16 = 16%**

[Probability of Exclusion =  $1 - \text{PI} = 1 - 0.16 = 0.84$   
=84%]

# The DNA Profiling Era



National Library of Medicine

# mixtures-older DNA technology

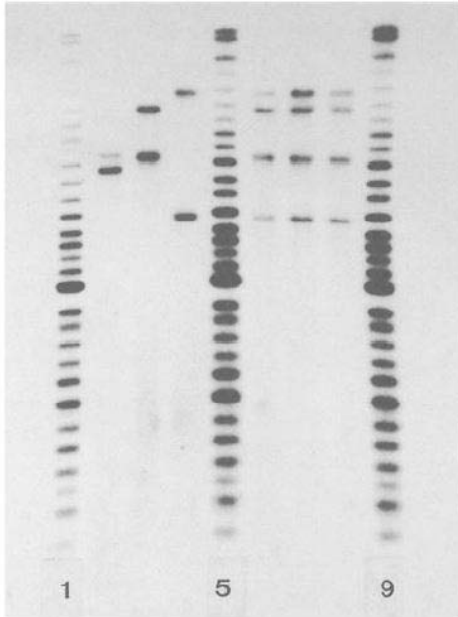


FIG. 10—DNA profile results for the genetic locus *D1S7* for stains containing blood from two sources (Lanes 6, 7, and 8). The mixed stains contained 25  $\mu$ L of blood from the female donor and 50  $\mu$ L of blood from the male donor. Lanes 1, 5, and 9 contain size markers. Lane 2 is the K562 cell line control. Lane 3 and 4 are the female and male donor controls, respectively.

Adams, *JFS* 36 1991:1284-1298

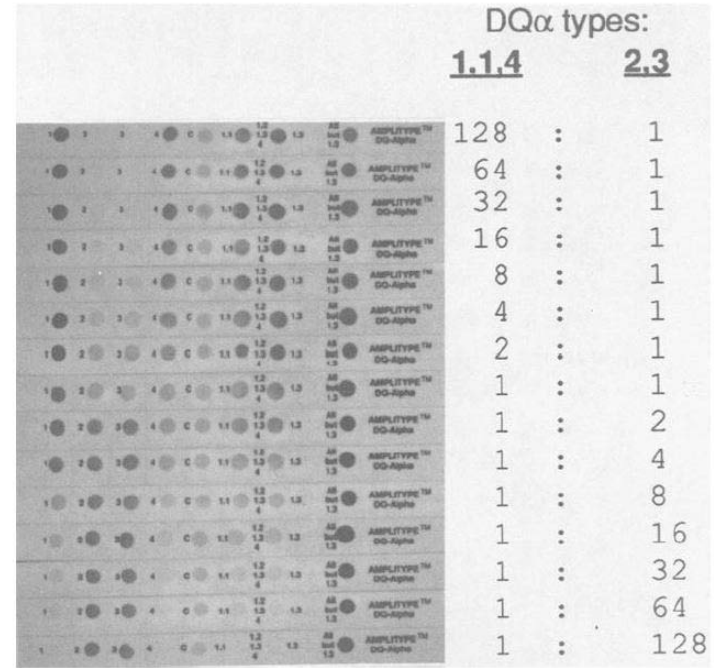


FIG. 2—A 50-ng mixture: DQ $\alpha$ 1,4 DNA and DQ $\alpha$ 2,3 DNA were mixed in the proportions indicated above. For each sample, a total of 50 ng of this DNA mixture was added to the PCR mix. The samples were amplified for 32 cycles, and DQ $\alpha$  typing was performed as described in **Materials and Methods**. As the quantity of DNA corresponding to the minor component genotype is decreased relative to the major component genotype, the resulting dot intensity for the minor component decreases relative to the major component.

Blake, E., *JFS* 37 1992, 700-26

# 1995



OJ Simpson grimaces as he tries on a glove during his murder trial. Photograph: PA

**a, Profiles from Bundy**

Item	Description	Number of loci		
		RFLP	PCR	Not excluded
42	NB - pool		1	NB
47	1st drop by victims		7	OS
48	Bundy walk		7	OS
49	Bundy walk		6	OS
50	Bundy walk		7	OS
52	Bundy walk	5	7	OS
56	Shoe print		5	NB
78	RG boot drop	5	6	NB, RG
84	NB nails		7	NB
115	Rear gate		2	OS
116	Rear gate		2	OS
117	Rear gate	9	2	OS

**e, Profiles from the Rockingham glove**

Item	Description	Number of loci		
		RFLP	PCR	Not excluded
9	Inside/back of wrist		1	NB, RG
9:G1	Inside/back index finger	5	2	NB, RG
9:G2	Inside/side middle finger	5	2	NB, RG
9:G3	Inside-back ring finger	8	2	RG
9:G4	Inside-back of hand	5	2	NB, RG
9:G9	Inside-by wrist notch		2	RG
9:G10	Inside-by wrist notch		2	RG, OS
9:G11	Outside-near wrist notch	1		NB, RG, OS
9:G12	Outside-near wrist notch	1		NB, RG
9:G13	Stitching on wrist notch	1		NB, RG, OS
9:G14	Inside-back of cuff edge	1		NB, RG

**b, Profiles from the Ford Bronco**

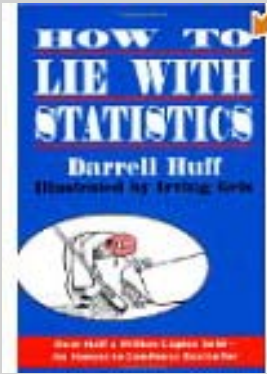
Item	Description	Number of loci		
		RFLP	PCR	Not excluded
23	Driver door interior		1	OS
24	Instrument panel		1	OS
25	Driver side carpet		2	OS
29	Steering wheel		6	OS, NB
30	Centre console		2	OS
31	Centre console		2	OS, RG
34	Driver side wall		1	OS
293	Driver side carpet		2	NB
303	Centre console	4*	2	OS, NB, RG
304	Centre console	4*	2	OS, NB, RG
305	Centre console	4*	2	OS, NB, RG

Combined 303, 304, 305. OS and RG not excluded.

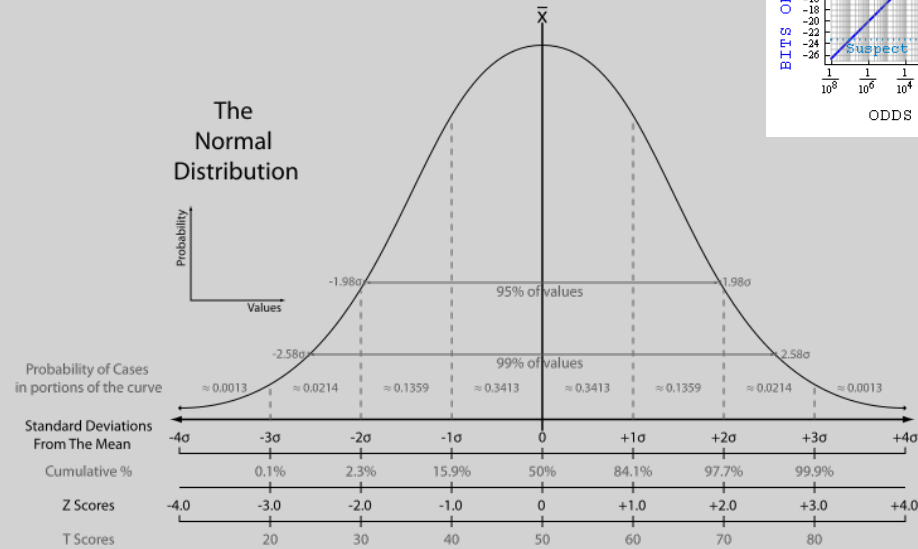
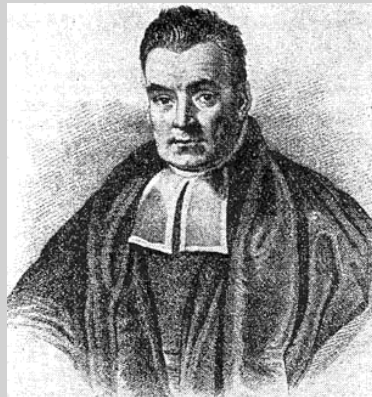
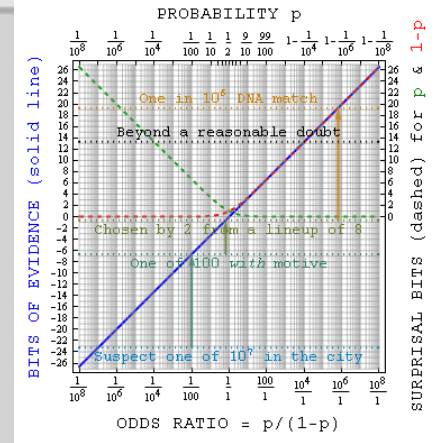
**d, Profiles from Rockingham socks**

Item	Description	Number of loci		
		RFLP	PCR	Not excluded
13	Ankle area 42A-1	14	7	NB
	Leg-opposite 42A-1		2	OS
	Leg-same side as 42A-1	9	2	OS
	Upper toe region 42A		2	OS
	Near ankle 42B		2	NB
	Near ankle 42B		2	NB

Bruce Weir: DNA Statistics in the Simpson Matter, Nature Genetics 11 365-368 (1995)



# The Science of Mixtures



“it is only the manipulation of uncertainty that interests us. We are not concerned with the matter that is uncertain. Thus we do not study the mechanism of rain; only whether it will rain.”

Dennis Lindley, "The Philosophy of Statistics", *The Statistician* (2000)



# *The Science of Mixtures*

## **Early Evangelism: Ian Evett, Home Office FSS**

- **1983- “What is the probability that this blood came from that person? A meaningful question?”** (Evett, *J For Sci Soc* 1983 23 p35-39)
  - Use of LR instead of coincidence probability as a logical framework for assessment of probity of genetic evidence
  - Single source
- **1987-” On meaningful questions: a two-trace transfer problem”** (Evett, *J For Sci Soc* 1987 27 p375-381)
- **1991- ”A guide to interpreting single locus profiles of DNA mixtures in forensic cases ”** (Evett, Buffery, Willott and Stoney, *J For Sci Soc* 1990 3 p41-47)
- **1998- “Taking account of peak areas when interpreting mixed DNA profiles ”** (Evett, Gill, Lambert, *J For Sci* 1998 43 p62-69)

# ***“a two-trace transfer problem” (1)***

**Uses conventional markers and phenotype (not allele) frequencies**

## **Example 1 (single donor)**

- **A crime has been committed by a man who left a bloodstain at the scene.**
- **The blood is typed using a polymorphic genetic marker that has a number of distinct phenotypes  $\gamma_1, \gamma_2, \gamma_3, \dots$  that occur in the general population with relative frequencies  $q_1, q_2, q_3, \dots$ .**
  - **The scientist has evidence F**
    - **the bloodstain is typed as  $\gamma_1$**
    - **the suspect is typed as  $\gamma_1$**
  - **C: the suspect committed the crime**
  - **$\sim C$ : the suspect did not commit the crime**

# ***“a two-trace transfer problem” (2)***

## **Example 2 (two donors)**

- A crime has been committed by a two men who both left a bloodstain at the scene.
  - The scientist has evidence F
    - the bloodstain is typed as  $\gamma_1$  and  $\gamma_2$
    - the suspect is typed as  $\gamma_1$
  - C: the suspect was one of two men who committed the crime
  - $\sim C$ : the suspect was not one of the two men who committed the crime
- Likelihood Ratio (LR) =  $\frac{\text{Probability of F given C is true}}{\text{Probability of F given } \sim C \text{ is true}}$

## ***“a two-trace transfer problem ” (3)***

- LR (example 1) =  $1 / q_1$
- LR (example 2) =  $(1 \times q_2) / 2 q_1 q_2 = 1 / 2q_1$
- Thus the LR is  $\frac{1}{2}$  that of the single stain case (evidence is less probative)
- If n different bloodstains of types  $\gamma_1, \gamma_2, \gamma_3 \dots \gamma_n$ , and a suspect of type  $\gamma_1$  then the LR =  $1/nq_1$  (i.e. reduction in LR dependent upon no. of different donors and, with the exception of  $\gamma_1$ , not on their relative phenotype frequencies)
- If q is greater than 0.5 then the LR would be less than 1!
  - Evidence reduces support for C versus  $\sim C$

# ***“interpreting single locus profiles of DNA mixtures”***

- Uses DNA markers and band (allele) frequencies
- Example 1 (four allele mixture, abcd with suspect possessing two of them, b and c)
  - $LR = 2f_a f_b / 24f_a f_b f_c f_d = 1/12f_b f_c$ 
    - One sixth of the LR obtained if only one assailant and bands b and c only)
  - $LR = 1/24f_a f_b f_c f_d$ 
    - If second suspect is arrested and he has a and b alleles)
- Example 2 (three allele mixture abc with suspect possessing two of them, b and c)
  - $LR = (f_a + 2f_b + 2f_c) / 12f_b f_c (f_a + f_b + f_c)$
- As no of alleles increases the LR evaluation becomes “quite complicated”
- Evidential strength falls rapidly with increasing numbers of alleles

# ***“taking account of peak areas when interpreting mixed DNA profiles”***

- **Conceptual paper that establishes logical framework for taking into account peak areas when interpreting mixed DNA profiles**
- **Use of peak area data and mixing ratios permits the ranking of different LRs that individually evaluate all possible combinations of genotypes present in the mixture**
- **Need computer programs**



Forensic Science International  
91 (1998) 55–70

**Forensic  
Science  
International**

## Analysis and interpretation of mixed forensic stains using DNA STR profiling

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## ***Analysis and interpretation of mixed forensic stains using DNA STR profile***

- **Step 1: identify the presence of a mixture**
- **Step 2: identify the number of contributors**
- **Step 3: determine the approximate 'ratio' of the components in the mixture**
- **Step 4: determine the possible pairwise combinations for the components of the mixture**
- **Step 5: compare the resultant profiles for the possible components of the mixture with those from the reference samples**



- **Step 1: identify the presence of a mixture**
  - Extra bands
    - Also should distinguish true second donor alleles from stutter, chromosomal abnormalities, pullup,  $n + 1$  bands
  - Allele peak asymmetry
    - Also should distinguish true second donor alleles from differential amplification of the alleles (e.g. stochastic effects and primer binding site mutations)
- **Step 2: identify the number of contributors**
  - Maximum alleles at a locus is 4 for two person mixture
  - 5 or 6 alleles indicative of three or more contributors
  - Experience indicates majority of mixtures encountered in casework are two person mixtures

- **Step 3: determine the approximate 'ratio' of the components in the mixture**

Mixture ratio		Dosage of alleles observed		Ratio of peak areas X:Y
Male (XY)	Female (XX)	X	Y	X:Y
10	1	12	10	1.2:1
5	1	7	5	1.4:1
4	1	6	4	1.5:1
3	1	5	3	1.6:1
2	1	4	2	2:1
1	1	3	1	3:1
1	2	5	1	5:1
1	3	7	1	7:1
1	4	9	1	9:1
1	5	11	1	11:1
1	10	21	1	21:1

- **Step 4: determine the possible pairwise combinations for the components of the mixture**

Four alleles (a,b,c,d)	Three alleles (a,b,c)	Two alleles (a,b)
a,b    c,d	a,a    b,c	a,a    a,b
a,c    b,d	b,b    a,c	a,b    a,b
a,d    b,c	c,c    a,b	a,a    b,b
<b>c,d    a,b</b>	a,b    a,c	a,b    b,b
<b>b,d    a,c</b>	b,c    a,c	<b>a,b    a,a</b>
<b>b,c    a,d</b>	a,b    b,c	<b>b,b    a,a</b>
	<b>b,c    a,a</b>	<b>b,b    a,b</b>
	<b>a,c    b,b</b>	
	<b>a,b    c,c</b>	
	<b>a,c    a,b</b>	
	<b>a,c    b,c</b>	
	<b>b,c    a,b</b>	

Key: bold entries represent reciprocal combinations.

“Using the quantitative information drawn from the peak areas in the profile and the approximate ratio of the mixture, some of the pairwise possibilities can then be discounted.”

- **Step 5: compare the resultant profiles for the possible components of the mixture with those from the reference samples**
  - If the profiles from the suspect's reference sample matches one or other of the alternatives, then that person cannot be eliminated as a possible contributor of one component of the mixed stain.
  - If the factual circumstances of a case are such that the profile from the donor of the sample might also be anticipated, then one might expect this individual's profile to complete the match and account for all of the remaining alleles.

## Later Evangelism: Bruce Weir, Dept Statistics, NCSU (now Univ Washington, WA)

- **1997- "Interpreting DNA mixtures"** (*Weir, Triggs, Stowell, Walsh, Buckleton, J For Sci 1997 42 p213-222*)
  - *refines and expands the LR concept and provides how- to formulations*
- **1999- "Interpreting DNA mixtures in structured populations"** (*Curran, Triggs, Buckleton, Weir J For Sci 1997 44 p987-995*)
  - *effects of population structure*
  - *role of evolution in shaping the probabilities of sets of profiles*
  - *Accounts for the information contained in the profiles of people who are declared not to have contributed to the evidence profile*

- **Post modern evangelism: taking into account PCR artifacts**
- **1998- “Interpretation of simple mixtures of when artefacts such as stutters are present-with special reference to multiplex STRs used by the Forensic Science Service”** (Gill, Sparkes, Buckleton, For Sci Int 1998 95 p213-224)
- **2009- “Interpreting low template DNA profiles”** (Balding, Buckleton, For Sci Int Genet 2009 4 p1-10)
- **2010- “A universal strategy to interpret DNA profiles that does not require a definition of low-copy-number”** (Gill, Buckleton, For Sci Int Genet 2010 4 p221-7)

# ***Meanwhile back at the RMNE Ranch***

- **1993 “Forensic inference from genetic markers”**  
(Devlin, 1993, Stat Meth Med Res 2 p241-262)
  - **how to calculate PE (PI)**

# 2009

*J Forensic Sci*, May 2009, Vol. 54, No. 3  
doi: 10.1111/j.1556-4029.2009.01046.x  
Available online at: www.blackwell-synergy.com

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Angelo Della Manna,<sup>2</sup> M.S.; Ann M. Gross,<sup>3</sup> M.S.; Richard A. Guerrieri,<sup>1</sup> M.S.;  
Jennifer C. Luttman,<sup>1</sup> M.F.S.; and David Lee McClure,<sup>4</sup> B.S.

## Mixture Interpretation: Defining the Relevant Features for Guidelines for the Assessment of Mixed DNA Profiles in Forensic Casework\*

**ABSTRACT:** Currently in the United States there is little direction for what constitutes sufficient guidelines for DNA mixture interpretation. While a standardized approach is not possible or desirable, more definition is necessary to ensure reliable interpretation of results is carried out. In addition, qualified DNA examiners should be able to review reports and understand the assumptions made by the analyst who performed the interpretation. Interpretation of DNA mixture profiles requires consideration of a number of aspects of mixed profiles, such as

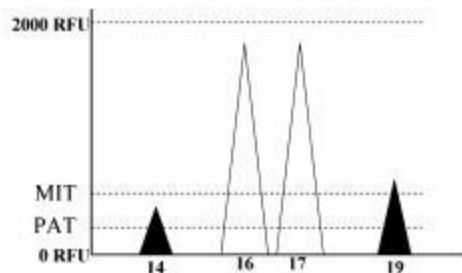


FIG. 1—Example of two person major and minor mixture profile. The minor contributor has one allele above the PAT but below the MIT and another allele above the MIT.

# Formalized the Use of Stochastic Threshold!



## ***Defining the Relevant Features for Guidelines for the Assessment of Mixed DNA Profiles***

- **“A standardized mixture interpretation protocol is not recommended or possible”**
- **Authors clearly prefer the random match probability and Probability of Inclusion (RMNE) (1-PE) approach instead of LR**
  - “convey to the trier of fact the probative value of the evidence in a straightforward fashion”

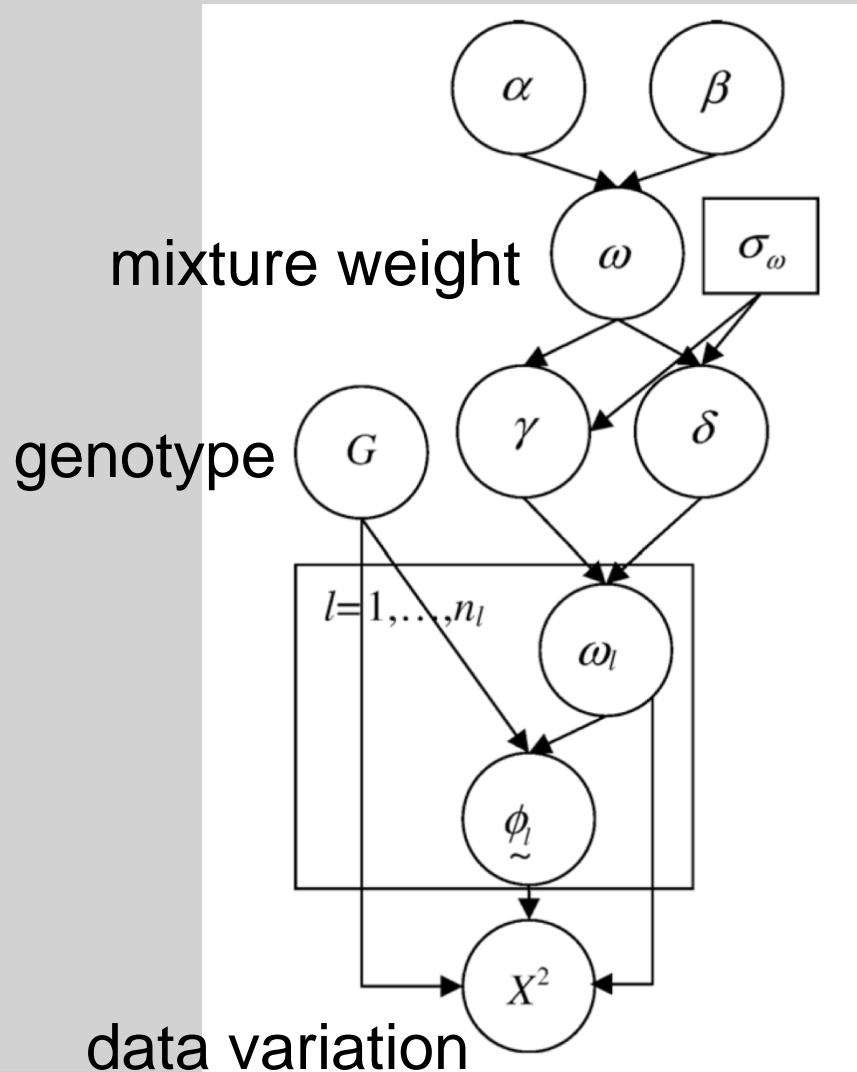
# ***Current evangelism: Quantitative Data Modeling (Mark Perlin, Cybergenetics)***

## **Hierarchical Bayesian Model with MCMC Solution**

- standard approach in modern science
- describes uncertainty using probability
- the "new calculus"
- replaces hard calculus with easy computing
- can solve virtually any problem
- well-suited to interpreting DNA evidence

*From Mark Perlin*

# Generally Accepted Method



James Curran. A MCMC method for resolving two person mixtures. *Science & Justice*. 2008;48(4):168-77.

# ***Software Solutions for Mixture Deconvolution?***

- **Linear Mixture Analysis (LMA)**

- *Part of TrueAllele system developed by Mark Perlin and Cybergenetics*
- *Perlin, M. W. and Szabady, B. (2001) Linear mixture analysis: a mathematical approach to resolving mixed DNA samples. *J.Forensic Sci.* 46(6): 1372-1378*

- **Least Squares Deconvolution (LSD)**

- *Described by T. Wang (University of Tennessee) at Oct 2002 Promega meeting*
- *Wang T and Birdwell JD (1996) Least-square deconvolution: a framework for interpreting short tandem repeat mixtures. *J.Forensic Sci.* 51(6): 1284-1297*

- **PENDULUM**

- *Part of FSS i-3 software suite*
- *Bill, M., Gill, P., Curran, J., Clayton, T., Pinchin, R., Healy, M., and Buckleton, J. (2005) PENDULUM-a guideline-based approach to the interpretation of STR mixtures. *Forensic Sci.Int.* 148(2-3): 181-189*

- **NYCOCME**

- *Statistical tool for mixture analysis using LR's and incorporating Pr (drop-in and drop-out) and LTDNA samples*

# Mixed DNA Profile

Frequentist approach

Bayesian approaches

**Method 1:**  
**Exclusion probability**

Random Man  
Not Excluded  
(RMNE)

Qualitative data

**Method 2:**  
**Qualitative approach**

Likelihood Ratio  
Approach

Quantitative and  
qualitative data

**Method 3:**  
**Binary model**

**Method 4:**  
**Continuous model**  
**MCMC**



Figure 7.1 from Tim Clayton and John Buckleton, Chapter 7 "Mixtures" in *Forensic DNA Evidence Interpretation* (2005) CRC Press

# *Community Effort and Diktats!*



# ***Technical Working Group on DNA Analysis Methods (TWGDAM)***

TWGDAM (1989) – *Crime Lab Digest* 16(2):40-59

Kearney *et al.* “Guidelines for a quality assurance program for DNA restriction fragment length polymorphism analysis” -**SILENT on MIXTURES**

TWGDAM (1991) – *Crime Lab Digest* 18(2):44-75

Kearney *et al.* “Guidelines for a quality assurance program for DNA analysis”

TWGDAM (1995) – *Crime Lab Digest* 22(2):20-43

Budowle *et al.* “Guidelines for a quality assurance program for DNA analysis”

# ***TWGDAM (1991) – “Guidelines for a quality assurance program for DNA analysis”***

- **4. Validation**
  - **4.1.5.5 Mixed Specimen studies-investigate the ability of the system to detect the components of mixed specimens and define the limitations of the system**
- **7. Analytical Procedures**
  - **7.1 Sample Evaluation and Preparation**
    - **7.1.2 When semen is identified, a method of differential extraction should be employed and, when appropriate, each of the DNA fractions typed**



# ***TWGDAM (1995) “Guidelines for a quality assurance program for DNA analysis”***

## **Identical to 1991 Guidelines**

- **4. Validation**
  - **4.1.5.5 Mixed Specimen studies-investigate the ability of the system to detect the components of mixed specimens and define the limitations of the system**
- **7. Analytical Procedures**
  - **7.1 Sample Evaluation and Preparation**
    - **7.1.2 When semen is identified, a method of differential extraction should be employed and, when appropriate, each of the DNA fractions typed**

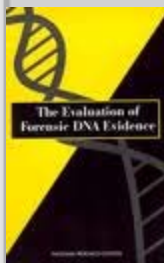
# *National Research Council Reports*

- 1992



“If a suspects pattern is found within the mixed pattern, the appropriate frequency to assign such a ‘match’ is the sum of the frequencies of all the genotypes that are contained within (i.e. that are a subset of) the mixed pattern” – RMNE

- 1996



in referring to the previous (1992) calculation, “this calculation is hard to justify, because it does not make use of some of the information available, namely, the genotype of the suspect. The correct procedure, we believe was described by Evett et al. (1991)” -LR

# DNA Advisory Board



# ***DNA Advisory Board Standards (1998)***

- **DAB created by the DNA Identification Act 1994**
  - Staffed and implemented 2005
- **QAS Standards for DNA Testing Laboratories**
  - Implemented October 1 2008
- **No substantive changes from TWGDAM Guidelines for mixtures**
  - **8. Validation**
    - **8.1.2.2 Species specificity, sensitivity, stability and mixture studies are conducted**
  - **9. Analytical Procedures**
    - **9.1.3 The laboratory shall have a procedure for differential extraction of stains that potentially contain semen**



**CURRENT ISSUE**

**BACK ISSUES**

**SEARCH  
ALL ISSUES**

**JOURNAL  
DESCRIPTION**

**EDITORS**

**INSTRUCTIONS  
FOR AUTHORS**

# Statistical and Population Genetics Issues Affecting the Evaluation of the Frequency of Occurrence of DNA Profiles Calculated From Pertinent Population Database(s)

DNA Advisory Board  
February 23, 2000

*Read about . . .*

# ***DAB Statistical and Population Genetic Issues***

## ***2000-Mixtures***

- **Mixtures are DNA samples derived from two or more contributors**
- **Evidenced typically by the presence of three or more peaks, bands, dots, and/or notable differences in intensities of the alleles for at least one locus in the profile**
- **In some situations, elucidation of a contributor profile is straightforward (e.g. DNA from an intimate swab revealing a mixture consistent with the composition of the perpetrator and the victim)**
- **When intensity differences are sufficient to identify the major contributor in the mixed profile, it can be treated statistically as a single source sample. At times, when alleles are not masked, a minor contributor to the mixed profile may be elucidated. Almost always in a mixture interpretation, certain possible genotypes can be excluded.**
- **It may be difficult to be confident regarding the number of contributors in some complex mixtures of more than two individuals; however, the number of contributors often can be inferred by reviewing the data at all loci in a profile.**

# ***DAB Statistical and Population Genetic Issues 2000-Mixtures (PE)***

- **When the contributors of a DNA mixture profile cannot be distinguished, two calculations convey the probative value of the evidence**
- **The probability of exclusion (PE) provides an estimate of the portion of the population that has a genotype composed of at least one allele not observed in the mixed profile**
  - **Knowledge of the accused and/or victim profiles is not used (or needed) in the calculation.**
  - **useful in complex mixtures, because it requires no assumptions about the identity or number of contributors to a mixture**
  - **the probabilities derived are valid and for all practical purposes are conservative. However, the PE does not make use of all of the available genetic data.**

# ***DAB Statistical and Population Genetic Issues 2000-Mixtures (LR)***

- **The Likelihood Ratio (LR) provides the odds ratio of two competing hypotheses, given the evidence**
- **a case of sexual assault for which the victim reported there were two assailants. A mixture of two profiles is observed in the "male fraction," and the victim is excluded as a contributor of the observed mixed profile. Two men are arrested, and their combined profiles are consistent with the mixture evidence**
- **A LR calculation logically might compare the probability that the two accused individuals are the source of the DNA in the evidence versus two unknown (random men) are the source of the evidence. Various alternate hypotheses can be entertained as deemed appropriate, given the evidence**
- **LR considers the identity and actual number of contributors to the observed DNA mixture**
- **LR makes better use of the available genetic data than does PE**





# Short Tandem Repeat (STR) Interpretation Guidelines

Scientific Working Group on DNA Analysis Methods  
(SWGDM)

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# ***STR Interpretation Guidelines-SWGDAM 2000***

## **3. Interpretation of Results**

- **3.1.1. *Single Contributor***
  - when the observed number of alleles at each locus and the signal intensity ratios of alleles at a locus are consistent with a profile from a single contributor
  - all loci should be evaluated in making this determination
- **3.1.2. *Mixtures With Major/Minor Contributors***
  - if there is a distinct contrast in signal intensities among the alleles. The difference is evaluated on a case-by-case context. All loci should be evaluated in making this determination
- **3.1.3. *Mixtures With a Known Contributor(s)***
  - when one of the contributors (e.g., the victim) is known, the genetic profile of the unknown contributor may be inferred.
  - This can be accomplished by subtracting the contribution of the known donor from the mixed profile
- **3.1.4. *Mixtures With Indistinguishable Contributors***
  - When major or minor contributors cannot be distinguished because of similarity in signal intensities or the presence of shared or masked alleles, individuals may still be included or excluded as possible contributors

# ***STR Interpretation Guidelines-SWGDAM 2000***

## **5. Statistical Interpretation**

**5.2. The formulas used in calculating the frequency of a DNA profile should be defined for the following:**

- 5.2.5. Mixture calculations**
  
- BUT HOW DO WE PERFORM THE CALCULATIONS?**
  - SILENCE IS GOLDEN?**



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## DNA commission of the International Society of Forensic Genetics: Recommendations on the interpretation of mixtures

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Recommendations on the interpretation of mixtures. *Forensic Sci. Int.* 160: 90-101



# ***Summary of ISFG Recommendations on Mixture Interpretation***

- 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**
- 7. 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**



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Editorial

## Editorial on the recommendations of the DNA commission of the ISFG on the interpretation of mixtures

“...**These recommendations have been written** to serve two purposes: to define a generally acceptable mathematical approach for typical mixture scenarios and to address open questions where practical and generally accepted solutions do not yet exist. This has been done **to stimulate the discussion among scientists in this field. The aim is to invite proposals and criticism in the form of comments and letters to the editors of this journal...We are hoping to continue the process to allow the DNA Commission to critically revise or extend these recommendations in due time...**”

# ***Responses to ISFG DNA Commission Mixture Recommendations***

- **UK Response**
  - Gill *et al.* (2008) *FSI Genetics* 2(1): 76–82
- **German Stain Commission**
  - Schneider *et al.* (2006) *Rechtsmedizin* 16:401-404 (German version)
  - Schneider *et al.* (2009) *Int. J. Legal Med.* 123: 1-5 (English version)
- **ENFSI Policy Statement**
  - Morling *et al.* (2007) *FSI Genetics* 1(3):291–292
- **Australia/New Zealand Support Statement**
  - Stringer *et al.* (2009) *FSI Genetics* 3: 144-145

# ***NIST MIX 05 Study***

LabID	Kits Used	Case1		
		Caucasians	African Americans	Hispanics
90	ProPlus/Cofiler	1.18E+15	2.13E+14	3.09E+15
34	ProPlus/Cofiler	2.40E+11	7.00E+09	9.80E+10
33	ProPlus/Cofiler	2.94E+08	1.12E+08	1.74E+09
6	ProPlus/Cofiler	40,000,000	3,500,000	280,000,000
9	ProPlus/Cofiler	1.14E+07	1.97E+07	1.54E+08
79	ProPlus/Cofiler	930,000	47,900	1,350,000
16	ProPlus/Cofiler	434,600	31,710	399,100

same epg: tremendous Inter-Lab variance



# ***July 2009 Rev. Quality Assurance Standards (QAS)***

## QAS Standard 5.3.2

**A casework CODIS administrator** shall be or have been a current or previously qualified DNA analyst ... with **documented mixture interpretation training**.

## QAS Standard 8.3.1

**Internal validation studies** conducted after the date of this revision shall include as applicable: known and non-probative evidence samples or mock evidence samples, reproducibility and precision, sensitivity and stochastic studies, **mixture studies**, and contamination assessment.

## QAS Standard 8.3.2

**Internal validation shall define quality assurance parameters and interpretation guidelines, including as applicable, guidelines for mixture interpretation.**

## QAS Standard 9.6.4

**Laboratories analyzing forensic samples shall have and follow a documented procedure for mixture interpretation that addresses major and minor contributors, inclusions and exclusions, and policies for the reporting of results and statistics.**

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### SWGDM Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories

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#### Scientific Working Group on DNA Analysis Methods (SWGDM)

The Scientific Working Group on DNA Analysis Methods, better known by its acronym of SWGDM, is a group of approximately 50 scientists representing federal, state, and local forensic DNA laboratories in the United States and Canada. During meetings, which are held twice a year, subcommittees discuss topics of interest to the forensic DNA community and often develop documents to provide direction and guidance for the community. A mixture interpretation subcommittee was formed in January 2007 and worked for several years to provide a guidance document on autosomal short tandem repeat (STR). This document was presented to the full SWGDM group and received approval in January 2010.

This document provides guidelines for the interpretation of DNA typing results from short tandem repeats (STR) and supersedes the Scientific Working Group on DNA Analysis Methods (SWGDM) Short Tandem Repeat (STR) Interpretation Guidelines (2000). The revised guidelines are not intended to be applied retroactively. Guidance is provided for forensic casework analyses on the identification and application of thresholds for allele detection and interpretation, and appropriate statistical approaches to the interpretation of autosomal STRs with further guidance on mixture interpretation. Laboratories are encouraged to review their standard operating procedures and validation data in light

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