

The Clayton Rules

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(slides adapted from John Butler)

(Basic Principles in Forensic DNA Evidence Interpretation, ISFG 2013, Melbourne)



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
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

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

Steps in the Interpretation of Mixtures (Clayton *et al.* 1998)

Step #1 Identify the Presence of a Mixture



Step #2 Designate Allele Peaks



Step #3 Identify the Number of Potential Contributors



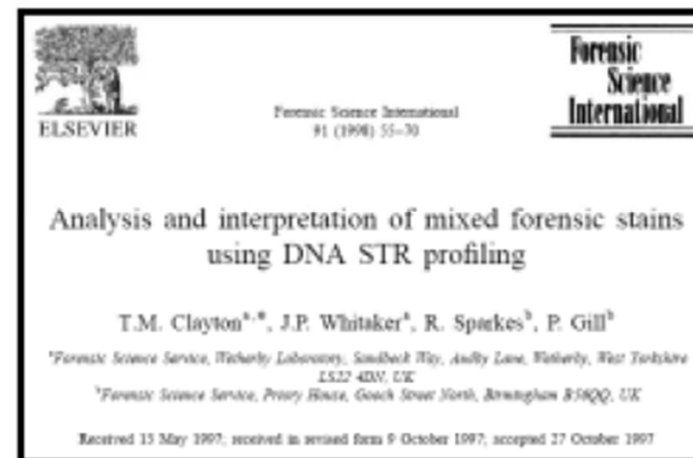
Step #4 Estimate the Relative Ratio of the Individuals Contributing to the Mixture



Step #5 Consider All Possible Genotype Combinations



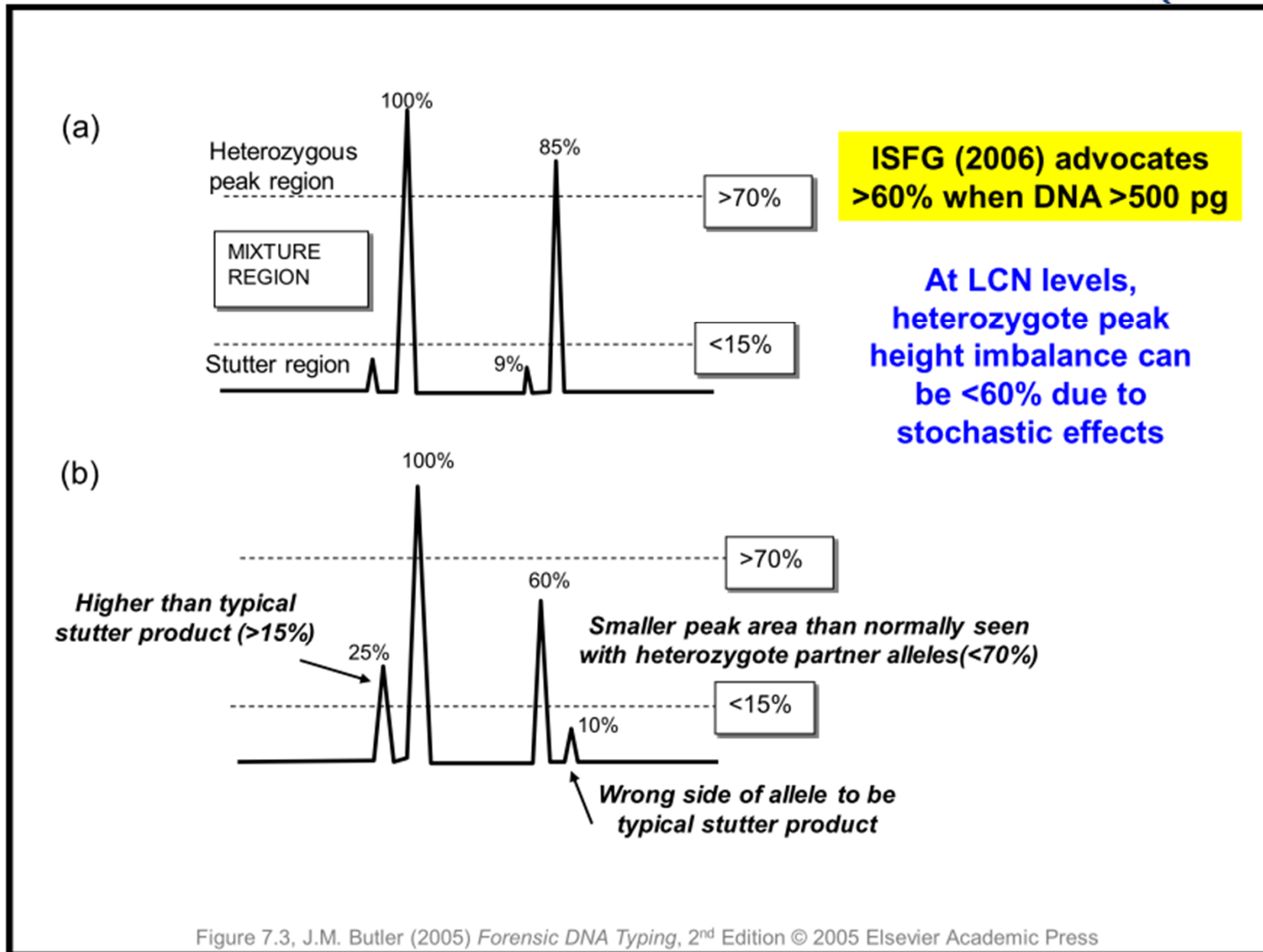
Step #6 Compare Reference Samples



Clayton *et al.* (1998) *Forensic Sci. Int.* 91:55-70

Step #1: Is a Mixture Present in an Evidentiary Sample?

- Examine the **number of peaks present** in a locus
 - More than 2 peaks at a locus (except for tri-allelic patterns at perhaps one of the loci examined)
- Examine **relative peak heights**
 - Heterozygote peak imbalance <60%
 - Peak at stutter position >15%
- Consider all loci tested



Step #2: Designate Allele Peaks

- Use regular data interpretation rules to decipher between true alleles and artifacts
- Use stutter filters to eliminate stutter products from consideration (although stutter may hide some of minor component alleles at some loci)
- Consider heterozygote peak heights that are highly imbalanced (<60%) as possibly coming from two different contributors

Step #3: Identifying the Potential Number of Contributors

- **Important for some statistical calculations**
- Typically if 2, 3, or 4 alleles then 2 contributors
- If 5 or 6 alleles per locus then 3 contributors
- If >6 alleles in a single locus, then >4 contributors

Step #4: Estimation of Relative Ratios for Major and Minor Components to a Mixture

- Mixture studies with known samples have shown that the mixture ratio between loci is fairly well preserved during PCR amplification
- Thus it is generally thought that the peak heights (areas) of alleles present in an electropherogram can be related back to the initial component concentrations
- Start with loci possessing 4 alleles...

Step #5: Consider All Possible Genotype Combinations

Table 3
Pairwise combinations of two, three and four alleles

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
c,d	a,b	a,b	a,c	a,b	b,b
b,d	a,c	b,c	a,c	a,b	a,a
b,c	a,d	a,b	b,c	b,b	a,a
		b,c	a,a	b,b	a,b
		a,c	b,b		
		a,b	c,c		
		a,c	a,b		
		a,c	b,c		
		b,c	a,b		

Key: bold entries represent reciprocal combinations.

Clayton *et al. Forensic Sci. Int.* 1998; 91:55-70

Step #6: Compare Reference Samples

- If there is a suspect, a laboratory must ultimately decide to include or exclude him...
- **If no suspect is available for comparison, does your laboratory still work the case?** (Isn't this a primary purpose of the national DNA database?)
- Victim samples can be helpful to eliminate their allele contributions to intimate evidentiary samples and thus help deduce the perpetrator

▫ Presentations from the ISFG workshops

Basic Principles in Forensic DNA Evidence Interpretation

Advanced Topics in Forensic DNA Evidence Interpretation

http://www.cstl.nist.gov/strbase/pub_pres/