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The effect of varying the number of contributors in the prosecution and alternate propositions

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Highlights

- One extra contributor under both H_p and H_a to fit the POI may overstate the *LR*
- One extra contributor under H_p but not H_a to fit the POI may be acceptable
- It takes considerable imbalance to favour an extra contributor
- The method of Slooten and Caliebe performs well

Abstract

Using a simplified model, we examine the effect of varying the number of contributors in the prosecution and alternate propositions for a number of simulated examples.

We compare the Slooten and Caliebe [1] solution, with several existing practices. Our own experience is that most laboratories, and ourselves, assign the number of contributors, $N = n$, by allele count and a manual examination of peak heights. The LR_n for one or a very few values is calculated and typically one of these is presented, usually the most conservative. This gives an acceptable approximation.

Reassessing the number of contributors if $LR = 0$ and adding one to N under both H_p and H_a to "fit" the POI may lead to a substantial overstatement of the *LR*.

A more reasonable option is to allow optimisation of the assignment under H_p and H_a separately.

We show that an additional contributor explained the single locus profile better when $PHR \geq 0.51$. This is pleasingly in line with current interpretation approaches.

Collectively these trials, and the solid theoretical development, suggest that implementation of the Slooten and Caliebe approach is optimal.

Keywords: DNA mixture interpretation, number of contributors, Slooten and Caliebe

Introduction

In forensic DNA interpretation the number of contributors to a mixture is strictly unknown. This is even true for apparently single sourced DNA samples. It is at least theoretically possible that there is an additional contributor whose alleles are masked or dropped out at all loci.

Recourse is usually taken to assigning a minimum number of contributors to a profile. This is viewed by many commentators, but not the authors, as a primary output of the DNA analysis and thought of as something that should be in the report to stakeholders [2].

In our experience the issue tends to relate to very small peaks and whether they are a trace contributor who is masked or dropped out at many allelic positions or whether such a peak is, for example; a large stutter, or a forward stutter. Practically a very minor trace contributor, if present, is unlikely to have much effect on the interpretation/resolution of the main donors' genotypes.

The subject of uncertainty in the number of contributors has been raised as an impediment to the effective interpretation of DNA evidence. Initially, arguments were raised around the fact that the number of contributors is unknown and may be different from that used in the interpretation. Recent court challenges have placed much emphasis on the possibility that the number of contributors used for analysis may be different from the 'true' number of contributors to the sample. For example, the following exchange from R v Trevean [SADC 419/2013]:

Q: *What's your opinion about how likely it is that there are more than four contributors to this mixed DNA sample*

A: *I have absolutely no idea and nor does [the prosecution witness]. The fact is that because we've got DNA peaks from people who are not there because they have dropped out, plus the fact that we have essentially all the peaks that are detectible anyway, it is not possible to say that you don't have more than four or more than five or more than six people.*

This was given impetus from papers [3] that showed that the number of contributors cannot readily be assigned by allele count alone. Superior to allele counting are likelihood methods [4-6]. These largely treat alleles as present or absent, that is, they do not currently account for height. They estimate the probability of the observed alleles given various numbers of contributors and account for allele probabilities and the coancestry coefficient. NOC*It* [7] adds a consideration of peak heights, and PACE [8] uses machine learning to assign probabilities to different numbers of contributors, and as such are likely to be the most informed tools.

It has been elegantly shown [9] that assigning probabilities to the number of contributors is superior to picking one value.

Before proceeding into this discussion it is necessary to consider what is meant by the number of contributors. Consider a mock sample, this is a sample constructed in the laboratory from DNA of known donors. We term this number of donors the **target number**. Next, imagine that three donors are used in the ratio 100:100:1. If we imagine that there is no discernible signal from the third contributor then this mixture could reasonably be termed a two donor mixture. Hence we could potentially define the **correct number** of contributors as the number of donors who contribute to recognisable signal. We have been unable to define the term recognisable signal and hence we suspect that the correct number cannot be known even in mock samples and never in casework. The last term we introduce is the **assigned number** of contributors. This is the number assigned by either an

operator or a software or both. There is evidence [10] that the assigned number may differ above or below the target number, but under a system of ideal interpretation, should equal the correct number.

In casework the target number, as well as the correct number, are unknown. The number of contributors assigned to a mixture is informed by that information that can reasonably be assumed. If, for example, it is reasonable to assume that the DNA of the victim and a consensual partner are present then these can be used to inform the assigned number. It is also often forgotten that defence and prosecution have every right to nominate numbers of contributors in their own propositions, but have no jurisdiction over the other party's choice.

When assigning a likelihood ratio, the probability of the evidence is evaluated under two exclusive propositions. One of these is typically aligned with the prosecution. The other is a rational alternative consistent with exoneration. We will term these H_p and H_a respectively. These propositions may be of the form:

H_p: POI is a donor to the mixture

H_a: POI is not a donor to the mixture

This is a departure from previous usage. In previous work we, and others, have used the sets H_p and H_d, or H_1 and H_2. The first set, H_p and H_d, has been criticised for implying that H_d is the proposition of the defense. In an adversarial environment the scientist is seldom in possession of the defense proposal. The set H_1 and H_2 avoids this implication but abandons any attempt to use the letter subscripts as cues to meaning.

Consider that the number of contributors (plausibly we mean here the correct number, termed N here) is unknown:

$$LR = \frac{\sum_n \Pr(N=n \mid H_p, I)\Pr(O \mid N=n, H_p, I)}{\sum_n \Pr(N=n \mid H_a, I)\Pr(O \mid N=n, H_a, I)}$$ where O is the observed evidence profile and I is the relevant background information. This recognises that the number of contributors considered under H_p and H_a may differ, most especially since H_p can legitimately assume that POI is present.

Let the set of all genotypes for n contributors be S^n which has elements S_j^n then

$$LR = \frac{\sum_n \Pr(N=n \mid H_p, I)\sum_j \Pr(O \mid S_j^n, N=n, H_p, I)\Pr(S_j^n \mid N=n, H_p, I)}{\sum_n \Pr(N=n \mid H_a, I)\sum_j \Pr(O \mid S_j^n, N=n, H_a, I)\Pr(S_j^n \mid N=n, H_a, I)} \quad \text{…equation 1}$$

This is a minor extension of a previously published equation [11]. In this paper, we look at the behaviour of this equation in a few situations.

Note that the number of elements in the set S^n can be very large. For each locus where there are 'a' possible alleles, a contributor can possess $\frac{a(a+1)}{2}$ different genotypes (obtained by the number of pairwise comparisons between a elements plus a homozygous genotypes). An n person mixture at l loci will possess $\left[a\left(\frac{a+1}{2}\right)\right]^{ln}$ possible genotypes sets, so if we take a modern multiplex that possesses approximately 20 loci, each with approximately 15 alleles, $J > 10^{41}$. For an N contributor set there are

n! orders of the genotypes. Many of these will not contribute to either one or both of the sums in the *LR* because:

1. $\Pr(\boldsymbol{O} \mid \boldsymbol{S}_j^n)$, is small relative to the probability of the profile given other elements in the set S^n, or
2. $\Pr(\boldsymbol{S}_j^n \mid H) = 0$, if the proposition requires the contribution of DNA from an individual whose genotype is not represented in set *j*.

It may be useful to think of the sum across *j* in the *LR* to be across all genotype sets where:

$$\Pr(\boldsymbol{O} \mid \boldsymbol{S}_j^n)\Pr(\boldsymbol{S}_j^n \mid H) > 0$$

However, it needs to be realised that the number of non-zero elements that would apply to the numerator and denominator could (and usually would) be different due to the second condition above being unique to each proposition. Therefore, it may be useful to think of *J* as the number of genotype sets for which $\Pr(\boldsymbol{O} \mid \boldsymbol{S}_j^n) > 0$, so that the sum is over the same number of genotype sets in numerator and denominator but may still possess some zero elements due to the second condition above. In the examples we simply omit these genotype sets from consideration.

Recently Slooten and Caliebe [1] published a result that is likely to very significantly advance this discussion. We reprise their finding here. Starting from equation 1, Slooten and Caliebe show that the overall *LR* is the weighted average of the LR_n values. LR_n is the *LR* value where the number of contributors is *n* under both H_p and H_a.

The weights for the weighted average are $\Pr(N = n \mid G_P, G_C, H_a)\dfrac{\Pr(N = n \mid H_p)}{\Pr(N = n \mid H_a)}$ where G_C is the profile of the crime stain and G_P is the profile of the person of interest. Since we consider H_a we can remove G_P from the conditioning yielding $\Pr(N = n|G_C, H_a)$. It is likely that only a few values of *n* need to be considered, maybe often only one or two.

If we assume that *n* is equally likely under H_p and H_a, specifically $\Pr(N = n|H_p) = \Pr(N = n|H_a)$ then the weights simplify to $\Pr(N = n \mid G_C, H_a)$. Note that the conditioning for $\Pr(N = n|H_p) = \Pr(N = n|H_a)$ does not contain G_C or G_P, and hence is informed only by whether or not the person of interest is a donor (but neither his profile nor the crime profile). This assumption is likely to be true or approximately true in the vast majority of cases. This is a remarkably useful finding and is the one we will examine here.

Slooten and Caliebe conclude: "*Thus, we believe that unless there are compelling case specific reasons to work with different values of the number of contributors under both hypotheses, the LR will be determined as a weighted average of LR(n) each with the same number n in the numerator and in the denominator*."

We use a simplified model (given in supplementary material) and brute force integration to provide numerical values for the terms $\sum_j \Pr(O \mid \mathrm{S}_j^n, N = n, H_p, I)$ and $\sum_j \Pr(O \mid \mathrm{S}_j^n, N = n, H_a, I)$ for equation 1.

We examine the performance of these equations in a very simple set of examples, cut down to the barest minimum of complexity to expose the underlying principles. For simplicity we assign the allele

probabilities p_{16} and p_{18} = 0.10 and use no adjustments for population co-ancestry. This allows us to examine some current practices and arguments regarding assigning the number of contributors.

Example 1.

Same assigned number of contributors under H_p and H_a

Consider the fictional one locus electropherogram, modelling analysis on an ABI 3130 capillary electrophoresis instrument, shown in Figure 1. Let the relevant background information be that this profile is from a semen stain on a sheet. A woman, V, alleges she was raped in her bed by one man and that she has no consensual partners. The sheet is from the bed on which she was raped. One man, P, is identified as a suspect. His genotype, G_P, is 16,18. The genotype of the complainant, G_V, is 14,20.

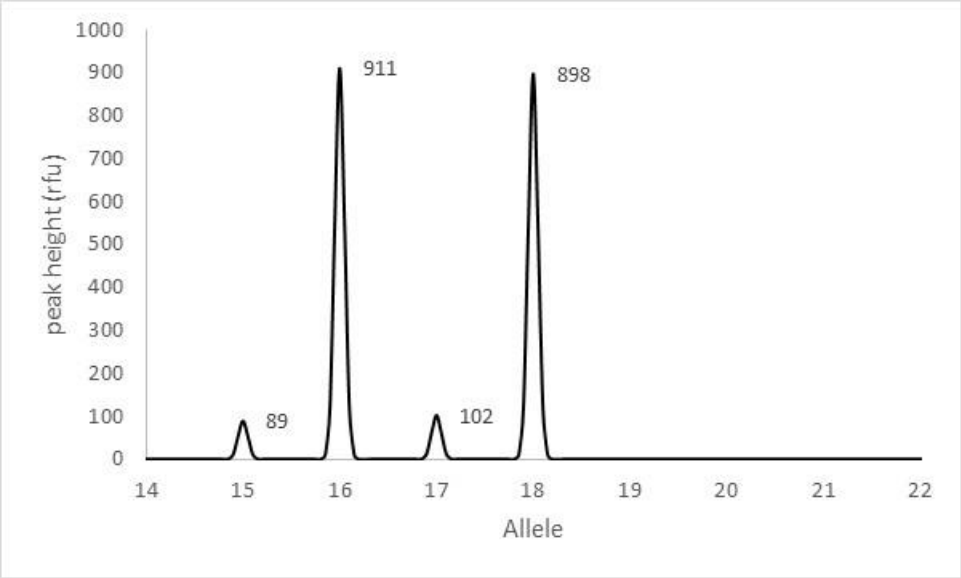

Figure 1. A depiction of one locus of an electropherogram.

The standard approach would be to examine all the loci in this profile with the knowledge of *I*, the relevant case circumstances, the genotype of V but not of P, and to assign the number of contributors as 1. This is equivalent to assigning N= 1 in equation 1.

Under H_p we specify that DNA from P is present. If N = 1 then this genotype composes the genotype set. Under H_a we specify that P is not present and if N = 1 there is one unknown donor who must be genotype 16,18. For this analysis the only set of genotypes that can explain the profile is 16,18. Hence there is one set S_1^1 = {16,18} and $\Pr(O \mid S_1^1 = \{16,18\}, N = 1)$ is the same under both H_p and H_a. Since P fully explains S_1^1 then $\Pr(S_1^1 \mid H_p) = 1$. Under H_a we require the unknown donor to be genotype 16,18 to complete S_1^1. This gives $LR = 50$ which is the standard answer for this problem.

Example 2.

Using the genotype of the accused to inform the number of contributors under H_p

Next, consider that we speculate that P is genotype 16,16. Now under H_p the accused is excluded if N = 1. However, P can be considered a contributor to the mixture if we increase N to 2. This feels very wrong, and has not been recommended by respected authorities due to concerns about contextual effects [2, 12]. In this paper we consider what would happen if the approach of using the accused's

profile under H_p was followed and compare it to the method of Slooten and Caliebe. We use the same electropherogram and background information given above. We assume $N = 2$ under H_p and $N = 1$ under H_a

Under H_p and $N = 2$ we assume the presence P and need another donor, U, who has the 18 allele. This suggests U is 18,18 or 16,18 or 18,Q where Q is any allele other than 16 or 18. There are two potential orders of the genotype of P and U.

Under H_a and $N = 1$ we assume the presence a donor who has the 16,18 genotype.

In Table 1 we give some of the terms and values used in Example 2. Under H_p then j = {16,16;18,18} or {16,16;16,18} or {16,16;18,Q}. Note that there are also the reverse orders of these {18,18;16,16} or {16,18;16,16} or {18,Q;16,16}. This ends up as a factor of 2 in the column $\Pr(S_j^2 \mid N=2, H_p)$. The terms $\Pr(O \mid S_j^n, N=n)$ in Table 1 are the probability of the observed peaks (at their observed heights), for the genotype set specified in the S_j^n column, and integrating across all values of mass parameter. The terms $\Pr(S_j^n \mid N=n, H)$ are the genotypic probabilities and the final column in Table 1 is the product of these two terms and when summed across all genotypes sets can be thought of as $\Pr(O \mid N=x, H)$.

Table 1. Some of the terms used in example 2. Q is any allele other than 16 or 18.

S_j^1	$\Pr(O \mid S_j^1, N=1)$	$\Pr(S_j^1 \mid N=1, H_a)$	$\Pr(O \mid S_j^1, N=1)\Pr(S_j^1 \mid N=1, H_a)$
16,18	1.16×10^{-10}	0.02	2.31×10^{-12}

S_j^2	$\Pr(O \mid S_j^2, N=2)$	$\Pr(S_j^2 \mid N=2, H_p)$	$\Pr(O \mid S_j^2, N=2)\Pr(S_j^2 \mid N=2, H_p)$
16,16;16,18	1.86×10^{-12}	0.02×2	7.43×10^{-14}
16,16;18,18	1.78×10^{-12}	0.01×2	3.55×10^{-14}
16,16;18,Q	4.81×10^{-23}	0.16×2	1.54×10^{-23}
$\sum_j \Pr(O \mid S_j^2, N=2)\Pr(S_j^2 \mid N=2, H_p) \rightarrow$			1.10×10^{-13}

Under H_a and $N = 1$ j = {16,18} and, $\sum_j \Pr(O \mid S_j^1, N=1)\Pr(S_j^1 \mid N=1, H_a) = 2.31 \times 10^{-12}$ Under H_p and $N = 2$ the three genotype sets yield $\sum_j \Pr(O \mid S_j^2, N=2)\Pr(S_j^2 \mid N=2, H_p) = 1.10 \times 10^{-13}$. This leads to:

$LR = \dfrac{1.10 \times 10^{-13}}{2.31 \times 10^{-12}} \approx 0.048 \ldots$equation 2. This is now not an LR on H_p and H_a but on the events $H_p \cap N = 2$ and $H_a \cap N = 1$

In Table 2 we calculate the probability of observing the trace profile assuming it has two unknown unrelated contributors.

Table 2. Some of the terms used in examples 2 and 3. Q is any allele other than 16 or 18.

S_j^2	$\Pr(O \mid S_j^2, N=2)$	$\Pr(S_j^2 \mid N=2, H_p)$	$\Pr(O \mid S_j^2, N=2)\Pr(S_j^2, N=2, H_a)$
16,16;16,18	1.86×10^{-12}	0.0004	7.43×10^{-16}
16,18;16,18	1.68×10^{-11}	0.0004	6.72×10^{-15}
16,18;18,18	1.86×10^{-12}	0.0004	7.44×10^{-16}
16,18;Q, Q	5.22×10^{-13}	0.0128	6.68×10^{-15}
16,Q;16,18	9.41×10^{-13}	0.0064	6.02×10^{-15}
16,18;18,Q	9.53×10^{-13}	0.0064	6.10×10^{-15}
16,16;18,18	1.78×10^{-12}	0.0002	3.55×10^{-16}
$\sum_j \Pr(O \mid S_j^2, N=2)\Pr(S_j^2, N=2, H_a) \rightarrow$			2.09×10^{-14}

We next try the Slooten and Caliebe solution (they have several but we mean the one selected in the introduction) to this example. We consider N = 1 or 2. Since P is excluded if N = 1 LR_1 = 0. The numerator of LR_2 for N = 2 is 1.10×10^{-13} (see table 1). The denominator of LR_2 for N = 2 is 2.09×10^{-14} (see table 2). This gives LR_2 = 5.32. We obtain $\Pr(N=1 \mid G_C, H_a) = 0.991$ and $\Pr(N=2 \mid G_C, H_a) = 0.009$ giving LR = 0.047. In contrast with the LR given in equation 2 the Slooten and Caliebe solution is an LR on H_p and H_a.

Example 3.

Speculating there are $N \geq n$ contributors under H_a.

Let the relevant background information be that this profile is from a semen stain on a sheet. A woman, V, alleges she was raped in her bed by one man and that she has no consensual partners. The sheet is from the bed on which she was raped. One man, P, is identified as a suspect. His genotype, G_P, is 16,18. Under H_p N = 1. However, we speculate that the defense wish to assert that N may be 1 or more.

In figure 2 we give heat maps of two of the two donor genotype sets. We have found these valuable to visualise the integration. The colors represent the relative probability density of the profile given the genotypes and templates that appear on the x and y axes. The left hand figure is for the genotype set 16,18;16,18. Since the genotypes are the same all that is needed is for the sum of the templates to be approximately 1,000 rfu. Hence we get a descending line of high density. The right hand figure is for the genotype set 16,16;18,18. To obtain a high density for the profile we need about 500 rfu of each donor (500 because they are homozygotes). The plots show that the combination 16,18;16,18 has a larger area of high density than 16,16;18,18 and this is reflected in the higher integral (see table 2).

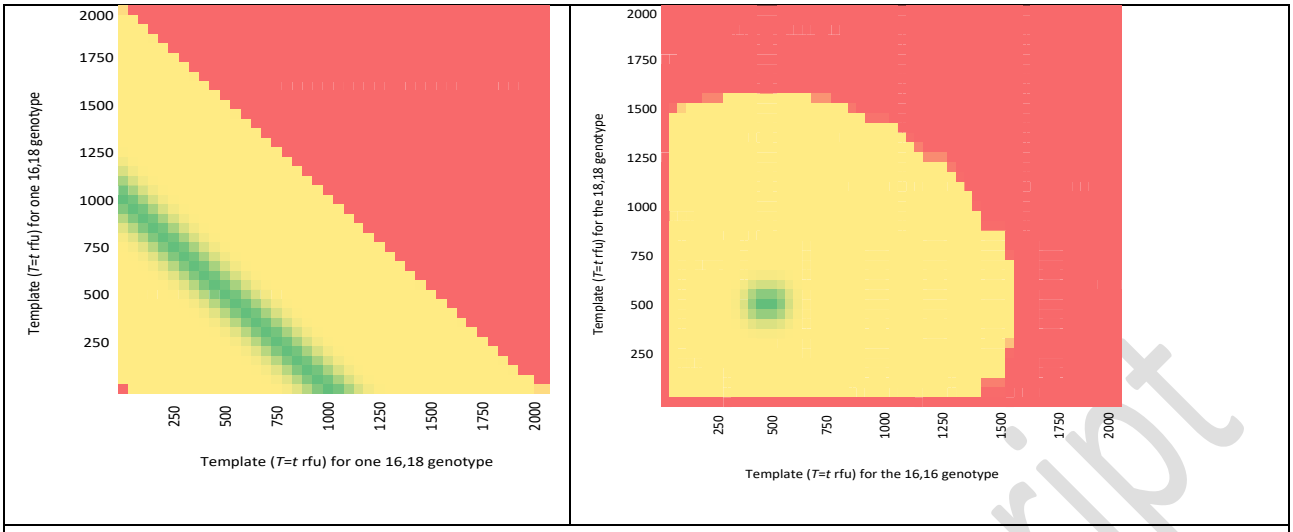

Figure 2. Heat maps of the probability density of varying contributor template amounts for each genotype for the two donor genotype sets. Left is the set 16,18;16,18 and right is the set 16,16;18;18. Green is high density, yellow is a mid-level density and red is low density. We only show these figures to represent the relative values of densities and so do not provide absolute values for colors.

$$LR = \frac{1.16\times10^{-10}}{2.31\times10^{-12}\underbrace{\Pr(N=1\,|\,H_a,I)}_{\text{prior that N=1 given } H_a} + 2.07\times10^{-14}\underbrace{\Pr(N=2\,|\,H_a,I)}_{\text{prior that N=2 given } H_a}}$$

This cannot be evaluated numerically without the priors on the number of contributors (marked in the equation). However given the relevant background information, I, it is likely that $\Pr(N=1\,|\,H_a,I) \geq \Pr(N=2\,|\,H_a,I)$. Equally, since $\Pr(N=1\,|\,H_a,I)+\Pr(N=2\,|\,H_a,I)=1$it is in the interests of the defense to assign $\Pr(N=1\,|\,H_a,I)=1$. This gives the standard answer for this problem (LR = 50).

In this analysis we have restricted H_p to N = 1. However the prosecution may wish to suggest that the number of contributors is at least 1 but not necessarily exactly 1. We consider N = 1 or 2. This suggests

$$LR = \frac{1.16\times10^{-10}\Pr(N=1\,|\,H_p,I)+6.76\times10^{-13}\Pr(N=2\,|\,H_p,I)}{2.31\times10^{-12}\Pr(N=1\,|\,H_a,I)+2.07\times10^{-14}\Pr(N=2\,|\,H_a,I)}$$

If we again make the unlikely but conservative assumption that $\Pr(N=1\,|\,H_a,I)=\Pr(N=2\,|\,H_a,I)=\Pr(N=1\,|\,H_p,I)=\Pr(N=2\,|\,H_p,I)$ we obtain LR = 49.8 (we are carrying more significant figures in the calculation, using the rounded numbers in table 2 gives 50.1)

We next try the Slooten and Caliebe [1] solution to this example. We consider N = 1 or 2. Example 1 gives LR_1 = 50. LR_2 is 32.7 and we obtain $\Pr(N=1\,|\,G_C,H_a)=0.991$ and $\Pr(N=2\,|\,G_C,H_a)=0.009$ giving LR = 49.8 but please note that Slooten and Caliebe [1] make the much more plausible assumption that $\Pr(N=1\,|\,H_a,I)=\Pr(N=1\,|\,H_p,I)$ and $\Pr(N=2\,|\,H_p,I)=\Pr(N=2\,|\,H_a,I)$.

Example 4.

These previous examples were applied to the profile in Figure 1 which is a perfect fit to a heterozygote with the stutter values used. In this example we vary the peak heights of the two allelic peaks and their stutters so that the peak height ratio (PHR) varies. This was done by moving the height of the 18 allele upwards but maintaining the total height of all four peaks at 2000 rfu by moving the height of the 16 peak downwards. The results are given in Figure 3.

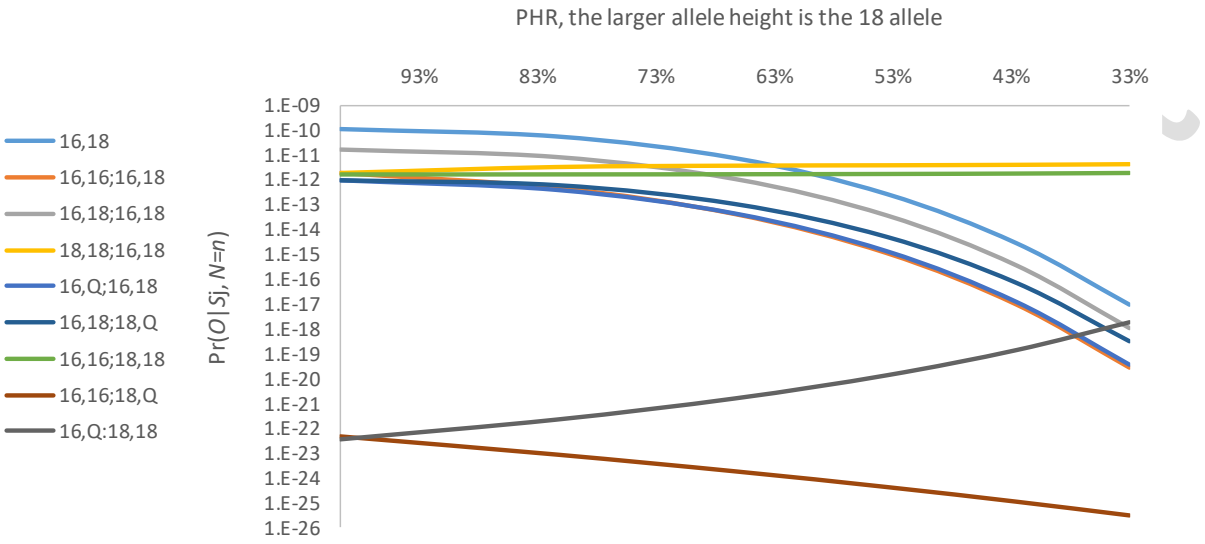

Figure 3. The behaviour of $\Pr(O \mid S_j, N = n)$ vs PHR

The $\Pr(O \mid S_j^n, N = n)$ usually trends downwards as PHR trends away from 1. The exceptions are the sets {18,18;16,18}, {16,16;18,18} and {16,Q;18,18} where the genotypes have the ability to adjust the mixture ratio of the two donors to fit the peak heights better to the observed data.

The values graphed in Figure 3 can be reprocessed to give the probability of the profile given the number of contributors $\sum_j \Pr(O \mid S_j^n, N = n) \Pr(S_j^n \mid N = n)$ (see figure 4). In this example the values for N = 1 and N = 2 become equal at PHR 51%. Recall that this depends on the modelling and the allele probabilities. However, it is pleasingly in line with experience.

At PHR = 51%, the trace is equally likely under N = 1 or N = 2, and hence the profile does not update the prior probabilities on N = 1 or N = 2 to new values. The *LR* for any suspect is the weighted average of LR_1 and LR_2 where the weights are the priors.

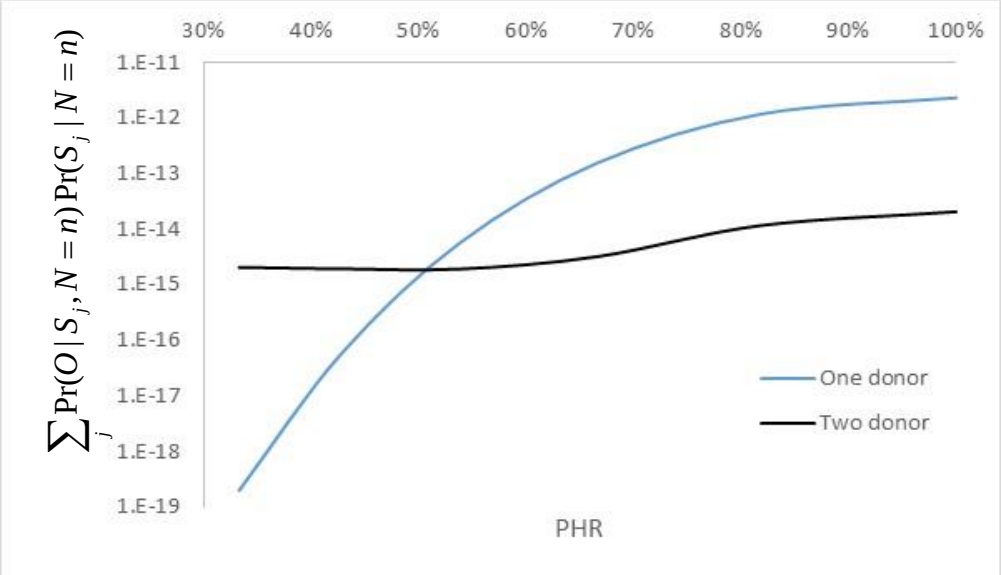

Figure 4. The behaviour of $\sum_j \Pr(O \mid S_j^n, N=n)\Pr(S_j^n \mid N=n)$ vs PHR for the one and two donor solutions.

Example 5.

Using the genotype of the accused to inform the number of contributors for a profile that does have some, but not conclusive, evidence supporting this.

We consider a three-locus profile. One locus has the peak heights shown in Figure 5 (termed the imbalanced locus) the remaining two loci have peak heights like those shown in Figure 1 (termed the balanced loci).

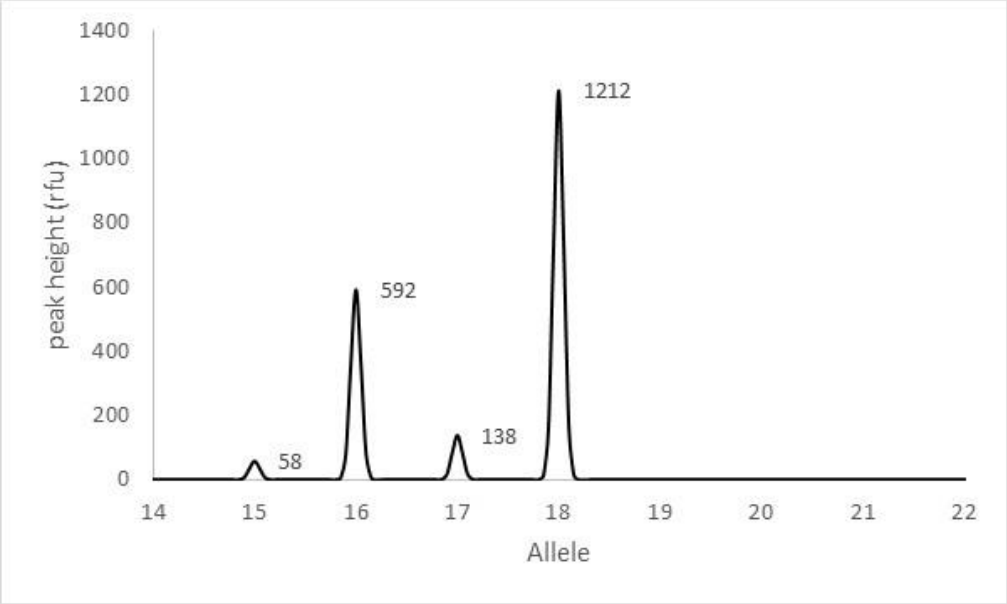

Figure 5. A depiction of one locus of an electropherogram showing imbalance, termed the imbalanced locus.

At the imbalanced locus P = 18,18. At the balanced loci P = 16,18.

We follow Slooten and Caliebe [1].

We work with the propositions:

H_p: The source(s) of DNA include POI

H_a: The source(s) of DNA include unknown individual(s) unrelated to POI.

POI is excluded if $N = 1$. If $N = 2$ POI helps the fit at the imbalanced locus. At the other two loci a two-donor solution is not needed to support H_p and POI neither helps nor hinders the fit. In Figure 6 we give the heat map of the probabilities for the combined three locus solution from Example 5 in order to visualise the integration. There is a general trend of $x+y$ =1,000 rfu for the preferred 16,18;16,18 solution at the two balanced loci. For the unbalanced locus, the templates are 650 and 1350 rfu. This solves to 16,18;18,18 650: 350 and 16,16;18,18 325:675. Some of the terms needed for this calculation are given in Table 3.

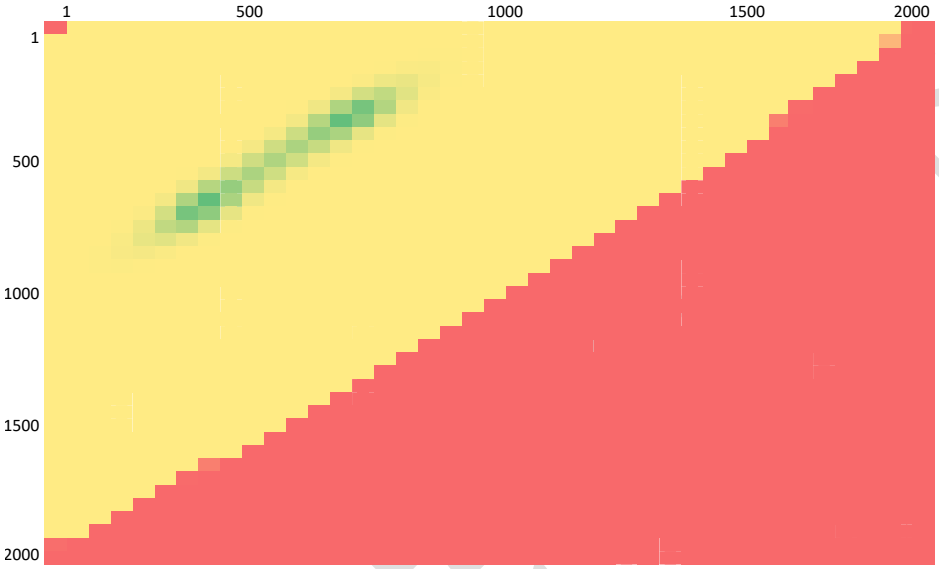

Figure 6. Heat map of Example 5, three locus solution following Slooten and Caliebe [1]. Red is area of relative low probability and green high probability.

Table 3. Some of the terms needed for example 5.

	$N = 1$	$N = 2$
$\sum_j \Pr(O \mid S_j^n, N = n) \Pr(S_j^n \mid N = n, H_p)$	0	3.12×10^{-34}
$\sum_j \Pr(O \mid S_j^n, N = n) \Pr(S_j^n \mid N = n, H_a)$	1.04×10^{-36}	2.78×10^{-39}
LR_n	0	1.12×10^{5}

$$\Pr(N = 2 \mid G_C, H_a) = \frac{2.78 \times 10^{-39}}{1.04 \times 10^{-36} + 2.78 \times 10^{-39}} = 0.002681$$

$$LR = 0.002681 \times 1.12 \times 10^{5} = 301$$

Conclusions

We compare the analyses above, and particularly the Slooten and Caliebe [1] solution, with several existing practices. We recognise that we have discussed the simplest situations such as one donor, one locus and at most three loci. This was to enable numerical integration and to expose the underlying principles. It would be advantageous to examine more complex situations. We hope to be able to report this soon.

Our own experience is that most laboratories, and ourselves, assign the number of contributors, $N = n$, by allele count and a manual examination of peak heights. This examination of peak heights may be informed by knowledge of characteristic variation of peaks height ratios, backward and forward stutter. The profile of any assumed contributors may be used in this evaluation but the profile of the POI, G_{p} should not be used. This value of $N = n$, is then used to calculate LR_n.

The weights for the weighted average suggested by Slooten and Caliebe [1] are $\Pr(N = n \mid G_C, H_a)$. As discussed above these weights are informed by the crime profile G_{C} but not the profile of the POI. The current practice described above is a manual assignment of this weight. However, to calculate LR_n one or a very few values must be chosen and typically one of these is presented, usually the most conservative, rather than a weighted average. This approach is shown in example 1. Example 3 allows a comparison of this practice with the suggestion of $N \geq 1$ under H_a. Current practice gives an acceptable approximation in this circumstance. The suggestion that $N = 2$ led to a significant overstatement of the *LR* for this example.

This fits with previous scholarship. Evett et al. [13] concluded that:

> *Provided the scientist has followed the guidelines and addressed propositions that are based on the number of contributors that best explains the questioned profile, then it is not to the advantage of the defendant to change the defence proposition to address a greater number of contributors.*

Similarly, Taylor et al. [14] carrying out the same process probabilistically conclude that:

> *… due to the slight favouring of simpler (lower contributor) models, there is still no advantage in artificially increasing the number of contributors to one or both of the hypotheses …*

and Budowle et al. [2] state:

> *… we stress that every effort should be made to provide the best estimate of the number of contributors. It is not in the best interest of the defense to suggest (an) unreasonable number of contributors; usually this will increase the LR favoring the prosecution's position.*

In example 4 we examined the probability of the profile under $N = 1$ and $N = 2$ as the PHR was varied away from 1. The $N = 2$ solution explains the profile better, for this model, when $PHR \geq 0.51$. This is pleasingly in line with current usage.

Our own preference is to stick with the outcome of the assignment made without knowledge of the genotype of the POI unless there is some very solid, unbiased, and supportable reason not to do so. However, we are aware that some laboratories will reassess if $LR = 0$ and often add one to N to "fit" the POI (termed $N = n+1$). In effect the maximum of LR_n and LR_{n+1} is reported. This practice can be examined using example 5. In that example $LR_1 = 0$ and $LR_2 = 1.12 \times 10^5$. The most reasonable assessment of the evidence for this example, we suggest, is the Slooten and Caliebe [1] weighted *LR*

value of 301. If only LR_2 is reported there is a significant chance of substantial overstatement of the *LR*.

A more reasonable option is to allow optimisation of the assignment under H_p and H_a separately. In example 2 this approach gives $LR \approx 0.048$ versus the Slooten and Caliebe [1] value of 0.047.

Example 5 can also be reprocessed into this approach. We obtain $LR = \frac{3.12\times10^{-34}}{1.04\times10^{-36}} = 302$ equation 3 (we are carrying more significant figures, the value 300 is obtained from the rounded values given in Table 3) for this approach versus the Slooten and Caliebe [1] value of 301. Again we assume that the value 302 may be reported. The *LR* for equation 3 is based on the propositions $H_p \cup N = 2$ and $H_a \cup N = 1$. The Slooten and Caliebe *LR* is based on H_p and H_a.

Allowing $N \geq 1$ under H_a and making the unlikely assumption that $\Pr(N=1 \mid H_a, I) = \Pr(N=2 \mid H_a, I) = \Pr(N=1 \mid H_p, I) = \Pr(N=2 \mid H_p, I)$ gives *LR* = 301. Recall that the Slooten and Caliebe [1] solution used here requires only the much more plausible assumption $\Pr(N=1 \mid H_a, I) = \Pr(N=1 \mid H_p, I)$ and $\Pr(N=2 \mid H_p, I) = \Pr(N=2 \mid H_a, I)$.

These trials, and the solid theoretical development of their publication, suggest the Slooten and Caliebe [1] approach performs well.

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References

[1] K. Slooten, A. Caliebe, Contributors are a nuisance (parameter) for DNA mixture evidence evaluation, Forensic Science International: Genetics.

[2] B. Budowle, A.J. Onorato, T.F. Callagham, A.D. Manna, A.M. Gross, R.A. Guerreri, J.C. Luttman, D.L. McClure, Mixture Interpretation: defining the relevant features for guidelines for the assessment of mixed DNA profiles in forensic casework, Journal of Forensic Sciences 54(3) (2009) 810-821.

[3] M.D. Coble, J.-A. Bright, J.S. Buckleton, J.M. Curran, Uncertainty in the number of contributors in the proposed new CODIS set, Forensic Science International: Genetics 19 (2015) 207-211.

[4] T. Egeland, I. Dalen, P.F. Mostad, Estimating the number of contributors to a DNA profile., International Journal of Legal Medicine 117 (2003) 271-275.

[5] H. Haned, L. Pène, F. Sauvage, D. Pontier, The predictive value of the maximum likelihood estimator of the number of contributors to a DNA mixture, Forensic Science International: Genetics 5(4) (2011) 281-284.

[6] H. Haned, L. Pène, J.R. Lobry, A.B. Dufour, D. Pontier, Estimating the Number of Contributors to Forensic DNA Mixtures: Does Maximum Likelihood Perform Better Than Maximum Allele Count?, Journal of Forensic Sciences 56(1) (2011) 23-28.

[7] H. Swaminathan, C.M. Grgicak, M. Medard, D.S. Lun, NOCIt: A computational method to infer the number of contributors to DNA samples analyzed by STR genotyping, Forensic Science International: Genetics 16 (2015) 172-180.

[8] M. Marciano, J. Adelman, PACE: Probabilistic Assessment for Contributor Estimation - A machine learning-based assessment of the number of contributors in DNA mixtures, Forensic Science International: Genetics 27 (2017) 82-91.

[9] A. Biedermann, S. Bozza, K. Konis, F. Taroni, Inference about the number of contributors to a DNA mixture: Comparative analyses of a Bayesian network approach and the maximum allele count method, Forensic Science International: Genetics 6(6) (2012) 689-696.

[10] J.-A. Bright, R. Richards, M. Kruijver, H. Kelly, C. McGovern, A. Magee, A. McWhorter, A. Ciecko, B. Peck, C. Baumgartner, C. Buettner, S. McWilliams, C. McKenna, C. Gallacher, B. Mallinder, D. Wright, D. Johnson, D. Catella, E. Lien, C. O'Connor, G. Duncan, J. Bundy, J. Echard, J. Lowe, J. Stewart, K. Corrado, S. Gentile, M. Kaplan, M. Hassler, N. McDonald, P. Hulme, R.H. Oefelein, S. Montpetit, M. Strong, S. Noël, S. Malsom, S. Myers, S. Welti, T. Moretti, T. McMahon, T. Grill, T. Kalafut, M. Greer-Ritzheimer, V. Beamer, D.A. Taylor, J.S. Buckleton, Internal validation of STRmix; A multi laboratory response to PCAST, Forensic Science International: Genetics 34 (2018) 11-24.

[11] J.S. Buckleton, J.M. Curran, P. Gill, Towards understanding the effect of uncertainty in the number of contributors to DNA stains., FSI genetics 1(1) (2007) 20-28.

[12] A.M. Jeanguenat, B. Budowle, I.E. Dror, Strengthening forensic DNA decision making through a better understanding of the influence of cognitive bias, Science and Justice 57(6) (2017) 415-420.

[13] I. Evett, S. Pope, Is it to the advantage of a defendant to infer a greater number of contributors to a questioned sample than is necessary to explain the observed DNA profile?, Science & Justice 54 (2014) 373-374.

[14] D.A. Taylor, J.-A. Bright, J.S. Buckleton, Interpreting forensic DNA profilig evidence without assuming a number of contributors, Forensic Science International: Genetics 13 (2014) 269-280.

Supplementary material

The model assumes a template, *T*, and no degradation. Hence the total allelic product for the 16 and 18 alleles are the same. The stutter ratio for allele a is *SR*a = 0.008 × -0.03a where a is the allele designation. This yields SR_{16} = 9.8% and SR_{18} = 11.4%. The expected peak height is set as $\frac{T}{1+SR_a}$ and the observed stutter as $SR_a \frac{T}{1+SR_a}$. The probability density for a stutter peak of observed height O_{a-1} and expected height E_{a-1} is modelled as $\log \frac{O_{a-1}}{E_{a-1}} \sim N\left(0, \frac{k^2}{\frac{b}{O_a}+O_a}\right)$. The probability density for an allelic peak of observed height O_a and expected height E_a is modelled as $\log \frac{O_a}{E_a} \sim N\left(0, \frac{c^2}{\frac{b}{E_a}+E_a}\right)$.

In this experiment k^2 = 10.45 and c^2 = 2.52 which are typical values for Identifiler Plus at 29 cycles of PCR on an ABI3130. *b* is set to 1,000.

The integrals of the type $\Pr(O \mid S_j, N=n) = \int_{T=0}^{7,000} \Pr(O \mid S_j, N=n, T=t) \Pr(T=t \mid N=n) dT$ are obtained by numerical integration. The prior on template $\Pr(T=t \mid N=n)$ is modelled as U[0,7000] for each of the *N* contributors.