Comment on “DNA Mixtures in Forensic Investigations: The Statistical State of the Art” by Julia Mortera


In the above article Mortera discusses the DNA evidence and the circumstances of the investigation/court proceedings in the case People v Hillary (New York). This is a case in which I was involved and of which I have first hand and documented knowledge.¹

Mr Oral (Nick) Hillary was charged and later acquitted of the murder of a 12 year old boy Garret Phillips, the son of his ex-girlfriend. The only physical evidence was a trace of DNA foreign to the victim from under the fingernails of the deceased. The analysis involved nine profiles from the same extract processed by the New York State Police (NYSP). There are three injections of each of three amplifications at 1, 1.5 and 2ng. The DNA was amplified using the reagent supplied in the Identifiler Plus™ PCR Amplification Kit (ThermoFisher Scientific, Waltham, MA, USA) which examines 15 loci. The amplification products were separated on a 3500 Genetic Analyzer (ThermoFisher Scientific, Waltham, MA, USA). The optimal input DNA for Identifiler Plus™ is 1ng.

Three of the nine profiles, one from each amplification selected without reference to Mr Hillary’s profile, were analysed using GeneMapper ID-X v1.5 (ThermoFisher Scientific, Waltham, MA, USA) at an analytical threshold (AT) of 50 rfu. The 50 rfu AT was set by validation studies carried out by the NYSP. This AT value of 50 rfu is at the low end of what I have seen used by a number of laboratories for this particular model of CE instrumentation. The output from this was analysed using the probabilistic genotyping software STRmix. STRmix results were precluded from the trial itself with His Honor ruling that the lack of internal validation at NYSP meant the results were not admissible. No DNA evidence at all was presented at trial.

Mortera makes a number of statements that warrant clarification.

Mortera states: “Nonetheless, based on the incriminating results given by STRmix, Hillary was indicted for murder on May 15, 2013” and “Recall that the results given by the analysis made with STRmix led to Hillary being indicted for murder”

The only references in the area of Mortera’s statements are to a New York times article and to Perlin’s blog page. Mark Perlin is the developer of TrueAllele, a competitor to the STRmix software. Neither of these references underpin the comment made by Mortera and the comments appear to be novel and developed by Mortera herself.

Mark Perlin initially worked with the prosecution. There is no report from Dr Perlin however he described his results in an email. An excerpt states: “while it is possible that Oral Hillary could have contributed his DNA to the sample, his contribution would be in the 1%-5% range, and is not statistically supported by the computer’s analysis of this data.”

¹ I have been asked to maintain the privacy of Mr Oral Hillary, acquitted of this murder, by not revealing his genotype and therefore will not present his profile data.
Subsequent to his work with the prosecution Perlin began advising the defense. At this time he began to describe potential exclusionary peaks between 30 and 50 rfu. I reference an email from the DA in this case, Bill Fitzpatrick to Perlin\(^2\).

STRmix results were generated well after the indictment. Garrett Phillips (aged 12) was murdered on October 24, 2011. The second indictment came on January 22, 2015. The DA in this case, Bill Fitzpatrick, first contacted me on November 2, 2015. Thus, the timeline alone indicates that the indictment was not influenced by the STRmix results as they came later.

Morera states “In the analysis made by STRmix, a threshold of 50 RFU was used, thus excluding the possibility that allele 18 at D3S1358 and at 13.2 for D19S433 are true alleles”

It is worth noting that the AT used was set by NYSP from their validation. It was not set by me. The CE analysis machine is a 3500 Genetic Analyzer (ThermoFisher Scientific, Waltham, MA, USA). 50rfu is low and 30rfu is in the noise on these machines.

Morera examines only the two loci mentioned above that come from one amplification. These have been taken from my webpage. There are \(9 \times 15 = 135\) loci in total. The two loci that appear on my webpage are selected because I suspect these are the ones that led Mark Perlin to speak about very low level exclusionary peaks that he had not mentioned whilst working for the prosecution.

Even the two loci chosen are not selected randomly but deliberately selected by me because they are the ones I think most support Dr Perlin’s view. I have approached Morera and suggested that we collaborate on this analysis and could have made available the fingernail profiles in full but not the genotype of Mr Hillary. This was not accepted, Morera citing work schedule and the time difference between Italy and New Zealand. Morera comments that “the full data were not made available to the author” but they could have been provided. Morera reports that she was asked to add the analysis of the murder of Garrett Phillips by a referee.

The 18 peak discussed by Morera has a peak height of 31 rfu and is similar in height to noise in the baseline around this locus. Morera’s interest in a peak at this height on a 3500 Genetic Analyzer, her labelling of it as an allele in the caption to Table 1, and her statement “It (table 1) gives the alleles present in the mixture, the corresponding peak heights, and the genotype of the victim” are concerning. The 13.2 peak discussed by Morera (also labelled allelic in Table 1) is due to pull-up from the 14 peak at the D3S1358 locus.

Below are the green and yellow dye channels (AT = 50rfu) from one of the nine electropherograms (epgs) (figure 1). Pull-up peaks in yellow can be seen right across the lane. The 13.2 at D19S433 is unlabelled as it is below the AT but can be seen as a small peak between the 13 and 14. Recognition of pull-up is inherent in interpretation of epgs since the inception of the use of fluorescent dye chemistry to label amplicons and thus is not novel, predating probabilistic genotyping. Failure to recognise or correctly manage artefacts is a common cause of false exclusions with probabilistic genotyping software. There is no evidence of managing such artefacts in Morera’s analysis of this case.

\(^2\) [https://johnbuckleton.wordpress.com/mark-perlin/]
Mortera states: “Furthermore, the two peaks in stutter position were probably defined as stutter peaks and not considered in the analysis.” From the subsequent discussion it is likely that Mortera is referring to the 12 and 14 peaks at the D19S433 locus. For clarification STRmix does consider all peaks in back and forward stutter positions and they are not removed for the analysis. Below are excerpts of the three most informative profiles at the D19S433 locus (see figure 2). The D3S1358 locus is included in the excerpts so that the reader can observe the pull-up.

As stated, back and forward stutter peaks are contained in the input file for STRmix. However it is valuable for subsequent discussion to consider whether the 12 and 14 peaks can be explained as stutter only. The following are some data for consideration:

Table 1. Peak heights and stutter ratio information for the D19S433 locus

<table>
<thead>
<tr>
<th>Peak</th>
<th>epg 1</th>
<th>epg 2</th>
<th>epg 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>71</td>
<td>63</td>
<td>80</td>
</tr>
<tr>
<td>12</td>
<td>173</td>
<td>168</td>
<td>225</td>
</tr>
<tr>
<td>13</td>
<td>2776</td>
<td>2891</td>
<td>3431</td>
</tr>
<tr>
<td>14</td>
<td>191</td>
<td>213</td>
<td>253</td>
</tr>
</tbody>
</table>
The peaks at 12 and 14 are almost the exact expected heights for stutter and there is certainly no reason to require an allelic component, although an allelic component could still be possible. However, as stated, these peaks were included in the STRmix analysis.

At this point please note that the major peaks are of a height in the order of 2,000-4,000 rfu. Please recall that these are thought to be amplifications of 1, 1.5, and 2ng on a 3500 genetic analyser. I would have expected peaks for these sized injections well above 15,000rfu for the major.

Morthera has amended the incorrect comments regarding indictment and stutter by taking down her article and reposting it 10th March 2020. She declined to rework the example to follow despite my presentation to her of much of the evidence in this note demonstrating the errors in DNAmixtures analysis. It would have been valuable to work more collaboratively and constructively on the comparison of software. Lawyers are likely to read sound bites from this article in court. Reasoned scientific discussion is unlikely in court and is much more likely to occur between scientists out of court.

At this point please note the presence of the small obligate trace allele at the 11 peak at the D19S433 locus. This can be seen in all the excerpts of this locus shown in Figure 2. There are 11 obligate trace alleles across the 9 replicates many of the obligate trace alleles appearing in multiple replicates.

The following is an excerpt from table 2 of the Mortera review article.

Table 2. An excerpt from Mortera’s table 2 for the D19S433 locus

<table>
<thead>
<tr>
<th>genotype</th>
<th>DNAmixtures</th>
<th>genotype</th>
<th>STRmix&lt;sup&gt;3&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 (victim allele),14 (victim allele)</td>
<td>0.214</td>
<td>11 (obligate trace),11 (obligate trace)</td>
<td>0.49</td>
</tr>
<tr>
<td>12(victim allele) ,13 (plausible stutter)</td>
<td>0.160</td>
<td>11 (obligate trace),13</td>
<td>0.21</td>
</tr>
<tr>
<td>13 (plausible stutter),14 (victim allele)</td>
<td>0.118</td>
<td>11 (obligate trace),15</td>
<td>0.19</td>
</tr>
</tbody>
</table>

Examination of table 2 shows that the DNAmixtures results are diagnosably incorrect. DNAmixtures has assigned weight for the trace to peaks corresponding with the victim’s alleles and stutter of those alleles. DNAmixtures has ignored the replicates obligate 11 allele. If you are going to ignore the obligate trace alleles why would you even treat this as a

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<sup>3</sup> This is the probability of the data given the genotype not the probability of the genotype given the data as implied by Mortera.
mixture at all [1]? I am hopeful that every competent DNA analyst reading this will note the obligate trace allele.

In correspondence Mortera has stated: “The example in question was given merely for illustrative purposes in order to show how sensitive results can be to model assumptions.” The statement that the answer will change if one changes the model is self-evidently true but completely unhelpful and, I suggest irresponsible, unless some analysis of the differences and especially which answer is more reasonable is made. This may be through ground truth known trials or by interpretation of simple scenarios where the answer may be predicted beforehand (for example single source profiles with no dropout [2]). It is of no value to the judicial process to know that answers differ, what is needed is to know which of the various models produces the more reliable output. Mortera did not need to use the highly sensitive murder of a 12 year old boy to achieve her stated goal. It would have been much better to use a mock sample where ground truth is known.

In regard to differing answers, STRmix is extensively case hardened, tested, validated, accredited, was run by experienced professionals on this case, the outputs were checked and agree with the expected results from the data.

This example is further proof that the process of probabilistic genotyping should be assessed by the analyst to ensure that the results (i.e. the epg and the probabilistic genotyping output) are intuitively concordant. One should not rely solely on software that give results such as ignoring the 11 allele as it is clear the 11 peak is an obligate allele for the minor (or trace) contributor. The STRmix result is subjectively correct and agrees with analyses performed using EuroForMix and LRmix.

I would like to renew my plea to Mortera to work constructively and collaboratively on this example, with some experienced examiner, so that corrected information may be put before the courts and community

John Buckleton

Thursday, 12 March 2020