Current challenges in statistical DNA evidence evaluation

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Chapter 4

An investigation of the potential of DIP-STR markers for DNA mixture analyses

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Abstract

The genetic characterization of unbalanced mixed stains remains an important area where improvement is imperative. In fact, using the standard tools of forensic DNA profiling (i.e., STR markers), the profile of the minor contributor in mixed DNA stains cannot be successfully detected if its quantitative share of DNA is less than 10% of the mixed trace. This is due to the fact that the major contributor's profile "masks" that of the minor contributor. Besides known remedies to this problem, such as Y-STR analysis, a new compound genetic marker that consists of a Deletion/Insertion Polymorphism (DIP) linked to a Short Tandem Repeat (STR) polymorphism, has recently been developed and proposed Castella et al. (2013). These novel markers are called DIP-STR markers. This paper compares, from a statistical and forensic perspective, the potential usefulness of these novel DIP-STR markers (i) with traditional STR markers in cases of moderately unbalanced mixtures, and (ii) with Y-STR markers in cases of female-male mixtures. This is done through a comparison of the distribution of 100,000 likelihood ratio values obtained using each method on simulated mixtures. This procedure is performed assuming, in turn, the prosecution's and the defence's point of view.

4.1 Introduction

The common way to analyse DNA mixtures for forensic purposes is to use the Polymerase Chain Reaction (PCR) and STR markers (Butler, 2011). One of the limitations of this method is that it does not work successfully if the proportion of the DNA quantities of the two contributors is more extreme than 1:10 (Clayton and Buckleton, 2005). Here, the threshold of 10% is retained as the limit of detection of the minor DNA for blood:blood mixtures. This value varies depending on the types of biological fluids which constitute the mixture and the specific combination of genotypes present in the mixture (as reported in (Applied Biosystems, 2012)) and should be assessed in the validation procedure (Butler, 2011). Mixtures with such extreme proportions are referred to in this paper as 'extremely unbalanced mixtures', opposed to 'moderately unbalanced mixtures', that are mixtures for which the proportion of DNA of each contributor is less extreme than 1:10. Situations involving extremely unbalanced mixtures are quite common, such as in cases of sexual assaults when the victim's DNA is largely predominant or cases of microchimerism during pregnancy (where minute quantities of fetal DNA are present in maternal blood). To address constraints implied by these kind of mixtures, Y-STR markers are widely adopted (Roewer, 2009), with the limitation that they provide information on the minor contributor only if that individual is male and the major contributor female. To address both the constraints of mixture imbalance and contributors' gender mismatch, an alternative analytical method has recently been developed and proposed (Castella et al., 2013). It is based on the use of new compound markers, each formed by an STR marker coupled to a marker in which a Deletion/Insertion Polymorphism (DIP) (Weber et al., 2002) is known to be present. So far a panel of 9 markers has been provided, called DIP-STR markers.

An object-oriented Bayesian network for the assessment of profiling results obtained with this novel technique has been developed (Cereda et al., 2014b). This network approach allows one to calculate a likelihood ratio for mixtures of two contributors, when the major contributor's genotype is known and the two competing hypotheses are 'the minor contributor is the suspect' (H_p) and 'the minor contributor is an unknown person, unrelated to the suspect' (H_d).

This paper aims to compare, from a statistical and forensic perspective, the potential usefulness of these novel DIP-STR markers (i) with traditional STR markers in cases of moderately unbalanced mixtures, and (ii) with Y-STR markers in cases of female-male mixtures. Section 4.2 starts with a brief introduction to the characteristics of the DIP-STR method along with the specification of the chosen STR and Y-STR marker systems. Next, Section 4.3 will present the interpretative model and the probabilistic tools (among which are graphical models) used to produce (through simulation techniques) likelihood ratio (LR) results for the three methods. Section 4.4 compares the distributions of the likelihood ratio results for DIP-STR and classical STR, and for DIP-STR and Y-STR. Section 4.5 focuses on the study of potential usability of the methods, that is the percentage of cases in which they are useful for the purpose of the investigation. The last Section 4.6 presents a discussion and conclusions, while the Appendix provides additional tables and figures.

4.2 Genetic background

This section briefly introduces the reader to the genetical background of DIP-STR markers. It also specifies the chosen STR and Y-STR marker systems. Particular features of the three methods, which are relevant for the understanding of the forthcoming sections, are also mentioned.

4.2.1 DIP-STR markers

DIP-STR markers were recently proposed as novel type of genetic markers (Castella et al., 2013). The novelty consists in pairing a Deletion/Insertion Polymorphism (DIP) (Weber et al., 2002) with a standard STR, to form a superlocus where the two composing loci are not independent because they are so close on the chromosomes (less than 500bp apart) that they cannot recombine, but independence can be assumed between the different DIP-STR markers. Two alternative allele-specific primers overlapping the DIP locus are designed, denoted L-DIP primer and S-DIP primer (L for *long* or S for *short*). Each of these is to be used together with a primer downstream the STR region. They are useful for mixtures of any unbalance proportion (DIP-STR genotypes of minor contributors were successfully typed at a ratio up to 1:1000 (Castella et al., 2013)) and where one contributor can be assumed as known, but they present a particular interest for extremely unbalanced mixtures, when the use of STR primers leads to masking of the minor contributor's genotype by the major contributor's genotype. This is due to the fact that the STR primers are loci specific. Two contributors necessarily have alleles from the same locus, although of possibly different lengths (i.e., repeat numbers), but STR markers do not differentiate between different alleles of the same locus in case of extremely unbalanced mixtures. In practice it is observed that annealing occurs mainly with those alleles that are present in predominant quantity, so that DNA of a minor contributor will not be successfully replicated. Due to the allele specificity of DIP-STR markers, DIP-STR genotyping allows the selected amplification of the unknown contributor's DNA, as long as it has alleles that are absent in the known contributor's genotype. For the purposes of this article, the known contributor is considered as the major one.

A first important feature of this set of markers concerns the exhaustiveness of the information that can be retrieved about the minor contributor, which depends on the combination of DIP alleles of the two contributors. This is why an initial step in the analysis consists in genotyping the major contributor's DNA, in order to know which DIP-primer to use for each locus of the mixture: if, at a particular locus, the major contributor is homozygous for the DIP alleles (i.e., S-S or L-L), the DIP-primer corresponding to the other DIP allele (L if the major contributor is S-S, S if the major contributor is L-L) will be used. Note that in case the major contributor is heterozygous for the DIP alleles (i.e., S-L), none of the DIP-primers is worth to be used at that particular locus. The best scenario is when the DNA of the major and the minor contributor are homozygous for different DIP alleles (i.e., one S-S and the other L-L, or viceversa). In this case, the possible results can show either two different minor DNA haplotypes or one, depending on the STR-homozygosity or heterozygosity of the minor contributor. On the other hand, when the major contributor is DIP-homozygous and the minor contributor is DIP-heterozygous, only one haplotype of the

minor DNA can be retrieved (i.e., the one with the DIP allele opposite to the DIP allele of the major contributor's DNA). A limitation of this method is that, when the predominant DNA is DIP-heterozygous or both contributors are DIP-homozygous of the same type, it is not possible to obtain any result from the analysis of the mixture, since both DIP primers (S and L), if used, will anneal to the major contributor's DNA. Table 4.1 summarises the possible outcomes. However, it is important to point out that even in those situations for which no alleles of the minor contributor are obtained, if the major contributor is DIP homozygous, some information about the minor contributor are nevertheless obtained, because it indicates that the minor contributor has the same DIP-homozygosity as the major contributor (both S-S or L-L).

DIP genotype of major contributor	**DIP genotype of minor contributor**	**DIP-STR results**
S-S	S-S	No results
	L-L	Complete genotype of the minor contributor
	S-L	Only the L DIP-STR allele
L-L	S-S	Complete genotype of the minor contributor
	L-L	No results
	S-L	Only the S DIP-STR allele
S-L	S-S	No results
	L-L	
	S-L	

Table 4.1: Informativeness of the different genotypic DIP-STR configurations. This table represents a single locus configuration, and the results in the last column are obtained using the DIP primer opposite to the DIP primer of the major contributor.

A first panel of DIP-STR markers,[1] was introduced in Castella et al. (2013): they are referred to in this paper as Marker 1, Marker 2, ..., Marker 9, respectively. Data from 103 unrelated Swiss individuals are used here for a Bayesian estimation of the allelic proportions at each of these markers. For further information on this method, see also Cereda et al. (2014b).

4.2.2 STR markers

STR markers are routinely used to genotype DNA traces (Butler, 2011). For the purpose of the current discussion, it is important to note that in case of extremely unbalanced mixtures, the use of STR markers generally does not allow one to be aware of the presence of a mixture, since the minor contributor's profile is masked by that of the major contributor.

[1]MID1013-D5S490, MID1950-D20S473, MID1107-D5S1980, rs11277790-D10S530, rs60194384-D15S1514, rs67842608-D5S468, rs66679498-D2S342, rs10564579-D3S1282, rs35708668-D5S2045

The 16 STR markers considered here are those of the kit AmpFℓSTR®NGM SElectTM NGMSelect^{TM}(Green et al., 2013). Data from 200 Swiss unrelated individuals will be used for a Bayesian estimation of the allelic proportions at each of these markers.

4.2.3 Y-STR markers

The term Y-STR locus designates an STR locus situated on the Y-chromosome (Butler, 2005). Y-STR markers are often used in forensic casework (e.g. Roewer, 2009; Roewer et al., 1992), in particular for their capacity to reveal male-specific Y-STR alleles in male/female DNA mixtures, even if extremely unbalanced. This makes them very useful in case of extremely unbalanced mixtures in which the major contributor is a female and the minor contributor is a male. However, in case the major contributor is male they are not useful.

Another drawback of the use of Y-STR markers is that the interpretation of Y-STR results is complicated by their haploidy and patrilineal inheritance, because male relatives will share the same Y-STR profile, even over several generations (if no mutations occur). Practically, this means that even in presence of a correspondence between the Y-STR profile of the crime stain and that of the suspect, his patrilineal relatives cannot be excluded as donors of the stain. Recently, a panel of 13 rapidly mutating (RM) Y-STR markers has been identified (Ballantyne et al., 2012), which successfully differentiates between closely and distantly related males. However, both the classical and the RM Y-STR techniques are useful only if the major contributor is a women and the minor contributor is a man.

It is important to mention that, due to the lack of recombination, Y-STRs form a single haplotype (i.e., the different markers cannot be considered independent).

The discussion presented in this paper refers to the PowerPlex® Y System Thompson et al. (2013). Data from 150 Swiss male unrelated individuals Haas et al. (2006) are used for a Bayesian estimation of the Y-STR haplotype proportions.

4.3 Interpretative model

Given a DNA mixture of two contributors, of which only one can be taken as known (say, the victim), and a suspect (available for comparative analyses) who shares alleles with the stain profile in some appropriate way, the two propositions of interest are typically addressed at 'source level' (Cook et al., 1998) and can be expressed as follows: H_p (usually referred to as the prosecution hypothesis), which asserts that the mixture originates from the victim and the suspect, and H_d (the defence hypothesis), which states that the mixed stain comes from the victim and an unknown person unrelated to the suspect. In order to assess the degree to which the profiling results allow one to discriminate between these two propositions, scientists should focus on the likelihood ratio, defined as follows:

$$LR = \frac{P(E \mid H_p, I)}{P(E \mid H_d, I)}. \tag{4.1}$$

This is a ratio of two probabilities P, where E represents the profiling results (i.e., the genotypes of the stain, of the victim and of the suspect) and I represents the background information (i.e., the circumstances of the case). The likelihood ratio (LR) is now widely considered the most appropriate framework to report on scientific evidence (Robertson and Vignaux, 1995; Aitken and Taroni, 2004). It provides a measure of the probative value of the finding given the proposition of interest. It is often convenient, due to the wide range of possible values, to convert them to the log-base-ten likelihood ratio. This paper will present a comparison of the log_{10} likelihood ratios obtained using the three different methods to simulated mixtures. Assumptions A1-A5, used for all the three methods, are listed below, while assumptions which are particular to a single method are specified in the corresponding sections.

A1 Each conceptual mixture is composed of the DNA of two contributors. The major contributor's genotype is available and known with certainty. This contributor is referred to as the victim.

A2 The DNA material is in sufficient quantity to obtain all the relevant genotypic information about the contributors that the considered set of markers is supposed to provide (i.e., no allelic drop-out.)

A3 There is no question of a close relative of the suspect being involved.

A4 No DNA artifacts (stutters or drop-in phenomena) occur during the analysis of the mixture.

A5 No subpopulation structures are taken into account.

The idea of the work reported here is to simulate, for each method, $n = 100,000$ mixtures of two contributors, under assumptions **A1** to **A5**, and to calculate the n likelihood ratios both assuming the prosecution's point of view and the defence's point of view. Thus, there is a total of $2n$ likelihood ratios. These values are stored, respectively, in vectors $\mathbf{LR_p}$ and $\mathbf{LR_d}$.

A mixture of two contributors is simulated through the random generation of the four alleles of the contributors, for each locus, with a probability based on the allelic proportions in the population of interest. The prosecution's point of view supposes that the two contributors, referred to as the victim V and the suspect S, are known. When the likelihood ratio for the proposition according to which the suspect is a contributor is calculated for such a mixture, a value greater than one is expected. The higher the likelihood ratio the more interesting is the chosen method from the prosecution's point of view. In this paper the distributions of the likelihood ratio obtained are used to compare the different methods with respect to the prosecution's point of view. Stated otherwise, LR_p is computed for H_p: 'The victim (V) and the suspect (S) contributed to the mixture (i.e., V+S)' and H_d: 'The victim (V) and an unknown person (U) contributed to the mixture (i.e., V+U)', when the mixture E is given by the alleles possessed by V and S.

When the defence's point of view is assumed, a person other than the suspect is considered as a contributor when simulating a mixture. If the suspect's genotype is compatible as a contributor to the mixture, a likelihood ratio higher than one is generally obtained. If the suspect's genotype is not compatible as a contributor to the mixture, a likelihood ratio of 0

is obtained. The higher the number of zero likelihood ratios which are obtained, the more attractive is the method from the defence's point of view. In summary, the defence's point of view is explored by (i) simulating mixtures involving the victim (V) and an unknown contributor (C_2, generated at random), and (ii) calculating likelihood ratios for a target proposition that specifies the suspect (different from C_2 and generated at random) as a second contributor. Again, discrete likelihood ratio distributions are obtained for the different methods, to be used for further comparative analyses. Stated otherwise LR_d is computed for H_p: 'The victim (V) and the suspect (S) contributed to the mixture (i.e., V+S)' and H_d: 'The victim (V) and an unknown person (U) contributed to the mixture (i.e., V+U)', when the mixture E is given by the alleles possessed by V and U. The next section offers details on this.

4.3.1 Likelihood ratios for STR markers

The mixtures which are simulated for the STR markers should all represent moderately unbalanced mixtures, otherwise the use of the STR method would not generally give any evidence of the presence of a second contributor. This means that another assumption should be introduced before evaluating the simulated STR results.

A6 for STR The mixture is moderately unbalanced.

To assess the results obtained from a moderately unbalanced mixture with the standard STR method, the likelihood ratio is calculated, marker by marker, using a Bayesian network proposed in Dawid et al. (2007) and Mortera et al. (2003). The overall likelihood ratio is obtained by the product of the marker specific likelihood ratios, due to the independence assumption made earlier in Section 4.2.2.

In order to simulate STR results for a mixture of two persons under assumptions **A1** to **A5**, four STR alleles (two for each contributor) are drawn, based on the allelic frequencies of the population of interest, for each marker. The first of the two contributors is defined as the victim, while the second is referred to as C_2. When considering the prosecution's point of view, the suspect is assumed to be C_2. Under the defence's point of view, the genotype of a third 'actor', which is the second contributor and is different from the suspect, has to be randomly generated. For each marker, a likelihood ratio is calculated using the Bayesian network, by specifying the alleles of the mixture, the genotype of the suspect and that of the victim. Doing so for all the markers, and multiplying the resulting likelihood ratios, the overall likelihood ratio for each mixture is obtained, depending on the particular point of view assumed (prosecution or defence).

If this process is iterated n times assuming the prosecution's point of view, a vector of n likelihood ratio results, called $\mathbf{LR_p^{STR}}$, is obtained and a discrete distribution for those values can be given. Iterating the process n times, assuming the defence's point of view, the vector $\mathbf{LR_d^{STR}}$ is obtained.

Prosecution's point of view

The $\log_{10}$ likelihood ratios obtained are all extremely high. The minimum value observed for the n simulated mixtures is 13.78, with a mean of 20 (see Table 4.3 for a detailed summary and comparison with the corresponding DIP-STR simulation results). The summaries for the distributions of the $\log_{10}$ likelihood ratios for each of the 16 STR markers are represented in the appendix (Table 4.8). The histogram for this distribution can be inferred from Figure 4.1 (grey bars (a), and grey line (b)).

Defence's point of view

When the defence's point of view is considered, most of the values of $\log_{10}\mathbf{LR_d^{STR}}$ are found to be zero. In fact, while marker specific likelihood ratios are occasionally higher than zero, the likelihood ratios over all markers are all found to be equal to zero (see Table 4.4 for a comparison with the corresponding DIP-STR simulation results). Thus, histograms are not very convenient to present these results, and a tabular summary appears to be more useful. Table 4.9 in the Appendix shows the percentage of values which are equal to 0 or which belong to one of the following intervals: $[1, 10)$, $[10, 100)$, $[100, 1000)$, $[1000, 10,000)$, $> 10,000$. For the interval [0,1] no likelihood ratio values are obtained. This is because, whenever the suspect's genotype is 'compatible' with the profiling results for the trace, the probability of observing the mixture profile given the first proposition (i.e., that the suspect is a contributor) is greater than given the alternative proposition (i.e., that an unknown person unrelated to the suspect is the second contributor). Thus likelihood ratios are either equal to 0, or greater than 1. Note that values greater than one, for this situation, wrongly support hypothesis H_p. For illustration, the bounds of the intervals shown in Table 4.9 are chosen to correspond to those of the scale of likelihood ratios and strength of verbal support in favor of the proposition H_p (Evett et al., 2000).

4.3.2 Likelihood ratios for DIP-STR markers

In Cereda et al. (2014b), an object-oriented Bayesian network (see Appendix, Figure 4.6) was constructed for the assessment of DIP-STR profiling results obtained from a mixture of two contributors (independently of the mixture proportion). This network allows one to obtain the likelihood ratio for the proposition according to which the suspect is the second contributor (versus the proposition that an unknown person is the second contributor), given the assumption that the first contributor is the victim.

The simulation of a mixture of two persons using DIP-STR alleles is similar to the procedure explained in Section 4.3.1. The only difference is that, for a given pair of contributors, possible results consist either of the DIP-STR allele(s) of the minor contributor (if the major contributor is DIP-homozygous and the minor contributor has at least one DIP allele of different kind), or of no alleles (see Table 4.1). As before, two vectors of n likelihood ratios are obtained, denoted here $\mathbf{LR_p^{DIP}}$ and $\mathbf{LR_d^{DIP}}$. Again these can be investigated through their discrete distributions.

Prosecution's point of view

The maximum $\log_{10}$ likelihood ratio observed for the simulated n mixtures is 13.71, which is close to the minimum value observed for the simulations using STR markers. However, for the DIP-STR simulations, the minimum value is 0 and the mean is 3.37 (see Table 4.3 for further summary statistics and a comparison with the results for the STR method). The summaries for the distributions of the $\log_{10}$ likelihood ratios for each of the 9 DIP-STR markers are represented in the Appendix (see Table 4.10). The histogram for this distribution can be inferred from Figure 4.1 (white bars (a), and white line (b)).

Defence's point of view

Table 4.11 in the Appendix represents the percentages of likelihood ratio results that fall into the various categories of probative value. Values equal to zero are obtained for 99.988% of all likelihood ratios (as shown by Table 4.4.)

4.3.3 Likelihood ratios for the Y-STR markers

The method for deriving the likelihood ratio for a mixture using Y-STR markers is different from that used for STR and DIP-STR markers Gill et al. (2001). Due to a lack of recombination, the majority of the Y-chromosome (including all the Y-STR markers currently used in forensic genetics) represents, in effect, a single locus Roewer (2009). Therefore, the independence assumption made for autosomal markers cannot be applied to estimate the population proportion for a Y-STR haplotype.

Moreover, the only situation in which the Y-STR analyses give interesting results is the one in which the major contributor is female and the minor one is male. This is why the following assumption is used for simulating Y-STR results.

A6 for Y-STR The known contributor to the mixture is female while the second, and incriminated one, is a man.

The simulations, in this case, consist in generating n times the Y-STR haplotype of the second contributor and of the suspect, with a probability based on the Y-STR haplotype proportion in the population of interest, and to evaluate the likelihood ratio, following Equation 4.2.

$$LR = \begin{cases} \frac{1}{\gamma_S} & \text{When assuming the prosecution's point of view} \\ \frac{a}{\gamma_{C_2}} & \text{When assuming the defence's point of view} \end{cases} \tag{4.2}$$

where γ_S and γ_{C_2} are, respectively, the population proportions of the Y-STR haplotypes of the suspect and of the actual second contributor to the mixture. Note that they are the same person under the prosecution's hypothesis. The parameter a is 0 every time the two haplotypes are different, otherwise it is 1.

Different approaches are currently available for assessing the rarity of particular Y-STR haplotypes, among which the counting method (Gill et al., 2001; Budowle et al., 2007), the

'haplotype surveying' method Roewer et al. (2000); Krawczak (2001), the k-method Brenner (2010), and the discrete Laplace method Andersen et al. (2013b). A Bayesian method, based on a uniform prior distribution, which is Dirichlet, is retained here to estimate the proportions of different Y-STR hayplotypes in a relevant population. The same method is used for estimating the population proportions of STR and DIP-STR alleles.

Table 4.2 represents the percentage of the different values of $\mathbf{LR_p^{Y-STR}}$ and $\mathbf{LR_d^{Y-STR}}$. Note that here the actual likelihood ratio values are used, instead of the $\log_{10}$, due to the limited extension of the range of values of the two vectors.

LR values	Percentage in $\mathbf{LR_p^{Y-STR}}$	Percentage in $\mathbf{LR_d^{Y-STR}}$
0	0	99.31
58.2	1.67	0.041
72.75	1.39	0.018
97	4.06	0.039
145.5	92.88	0.592

Table 4.2: Percentage of different values of $\mathbf{LR_p^{Y-STR}}$ and $\mathbf{LR_d^{Y-STR}}$.

Note that only four possible distinct likelihood ratio values (different from 0) are obtained. This is due to the fact that in the considered database (Haas et al., 2006), there are 4 different haplotypes which appear twice (and thus bring to a likelihood ratio of 97), one haplotype which appears three times (likelihood ratio of 72.75), one which appears four times (likelihood ratio of 58.2) while the other 135 different haplotypes appear only once each (likelihood ratio of 145.5). Likelihood ratios equal to zero are obtained, when using simulation for the defence point of view, each time the Y-STR genotype of the second contributor and of the suspect are not compatible.

The use of the assumption **A3** about the impossibility of kinship between the perpetrator and the suspect under the hypothesis H_d has a strong effect on the likelihood ratio values for Y-STR profiling results: in fact, as noted earlier, if no mutations occur, patrilineal relatives of the suspect share the same Y-STR profile, which would imply different values for the likelihood ratio if one takes them into account.

4.4 Comparison of the three methods

This section compares the DIP-STR method with both the regularly used STR method, and the Y-STR method. The comparison is based on the distributions of the likelihood ratio results obtained with the three methods, assuming the same point of view (i.e., of the prosecution, or of the defence).

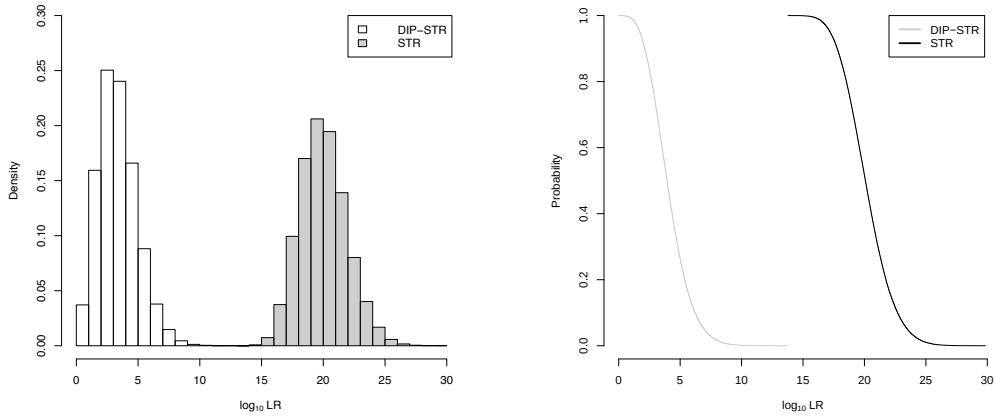
The comparison of the DIP-STR and the STR likelihood ratio results supposes moderately unbalanced mixtures because, otherwise, the use of STR markers is likely to miss any indication of the presence of a second person in the mixture. The comparison between the

DIP-STR and Y-STR likelihood ratio results assumes mixtures which could involve any unbalance proportion, but with the constraint that the major contributor is a women and the minor one is a man. Note that the latter comparison becomes relevant in case of extremely unbalanced mixtures, that is when STR markers can generally not be used.

The comparisons from the prosecution point of view are carried out by plotting in the same graph the histograms of the distributions of $\log_{10}\mathbf{LR_p}$ for the two methods, and in another graph their Tippett plots. The latter are graphical representations first reported for forensic DNA evaluation in Evett and Buckleton (1996), and inspired by the concepts of 'within-source comparison' and 'between-sources comparison' as defined by Tippett Tippett et al. (1968). In this kind of representation, the x axis represents the different ($\log_{10}$) values of the likelihood ratio from the prosecution point of view. The y axis represents the proportion of cases in which the likelihood ratio exceeds the corresponding value in the x axis.

4.4.1 Comparison of DIP-STR and STR assuming point of view of the prosecution

Before the comparison is performed in further detail, it is worth recalling that this is meaningful only under the assumption **A6 for STR**, that is in case of moderately unbalanced mixtures. Figure 4.1 represents the histograms and the Tippets plots for $\log_{10}$ of the likelihood ratio values for the two methods, assuming the prosecution point of view. Table 4.3 presents the standard summary statistics for the two distributions.



(a) (b)

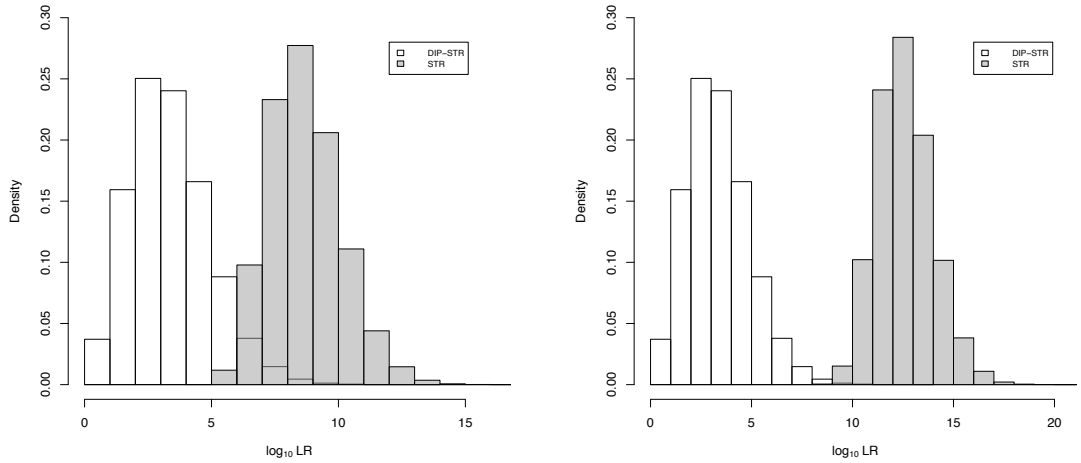
Figure 4.1: Graphical comparisons of the $\log_{10}\mathbf{LR_p^{STR}}$ and $\log_{10}\mathbf{LR_p^{DIP}}$ distributions in terms of superimposed histograms (a) and Tippett plots (b).

Figure 4.1 shows that the distribution of the likelihood ratio values for the STR markers is shifted towards higher values than the distribution of the likelihood ratio for the DIP-STR markers. This means that, from the prosecution's point of view, the use of the STR kit is more desirable. It has to be noticed, however, that since the STR kit has 7 markers more than the DIP-STR kit, this difference is little surprising.

Marker system	Min	1st Quantile	Median	Mean	3rd Quantile	Max
DIP-STR	0	2.228	3.201	3.367	4.324	13.706
STR	13.781	18.658	19.899	19.996	21.218	29.85

Table 4.3: The summaries of the distributions of $\log_{10}\mathbf{LR_p^{STR}}$ and $\log_{10}\mathbf{LR_p^{DIP}}$.

In order to arrange a comparison using the same number of markers for the two methods, 9 STR markers were chosen here out of the 16. There are 11,440 combinations of 9 markers out of 16, but here we have focused on the two combinations of 9 markers for which the means of the distributions of the $\mathbf{LR_p^{STR}}$ are, respectively, minimally and maximally separated of the distribution of the $\mathbf{LR_p^{DIP}}$. These two combinations have been found empirically, running simulations for each of combinations. Figure 4.2 shows the histograms for the distribution of $\log_{10}\mathbf{LR_p}$ for the two methods, using these two combinations of 9 out of 16 STR markers in comparison with the histogram for the distribution of $\log_{10}\mathbf{LR_p^{DIP}}$. Table 4.4 shows the summaries for the 3 distributions. These results confirm the previous finding: the STR markers system performs better than the DIP-STR marker system.



(a) (b)

Figure 4.2: Comparisons of the distribution of $\log_{10}\mathbf{LR_p^{DIP}}$ (white bars) with the distribution of $\log_{10}\mathbf{LR_p^{STR}}$ (grey bars) for the combination of markers for which the mean of the two distributions are (a) minimally and (b) maximally separated.

Marker system	Min	1st Quantile	Median	Mean	3rd Quantile	Max
DIP-STR	0	2.228	3.201	3.367	4.324	13.706
STR (min. separated)	4.800	7.653	8.557	8.677	9.578	16.140
STR (max. separated)	8.905	12.530	13.500	13.600	14.560	21.620

Table 4.4: Summaries of the distribution of $\log_{10}\mathbf{LR_p^{DIP}}$ and $\log_{10}\mathbf{LR_p^{STR}}$ choosing the 9 STR markers for which the means of the distributions are the minimally (second row) and the maximally (third line) separated.

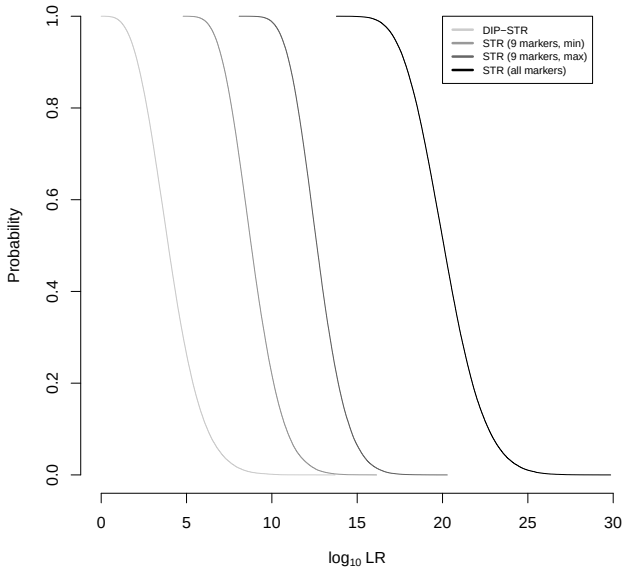


Figure 4.3: Tipett plots of the $\log_{10}\mathbf{LR}_{\mathbf{p}}^{\mathbf{DIP}}$ and $\log_{10}\mathbf{LR}_{\mathbf{p}}^{\mathbf{STR}}$ results for both, full STR profiles and profiles with reduced numbers of markers.

Figure 4.3 shows the Tippett plots of the DIP-STR $\log_{10}$ likelihood ratio distribution and the 3 different distributions of STR $\log_{10}$ likelihood ratios (i.e., one for full STR profiles, and two with only 9 markers). As may be seen, the two likelihood ratio distributions with 9 STR markers are closer to the DIP-STR likelihood ratio distribution than the one with 16, just as expected.

4.4.2 Comparison between DIP-STR and STR marker systems assuming the point of view of the defence

Tables 4.4 summarises the percentage of values of $\mathbf{LR}_{\mathbf{d}}^{\mathbf{STR}}$ and $\mathbf{LR}_{\mathbf{d}}^{\mathbf{DIP}}$ that fall into the different intervals of likelihood ratio values, corresponding to different expressions of probative strength.

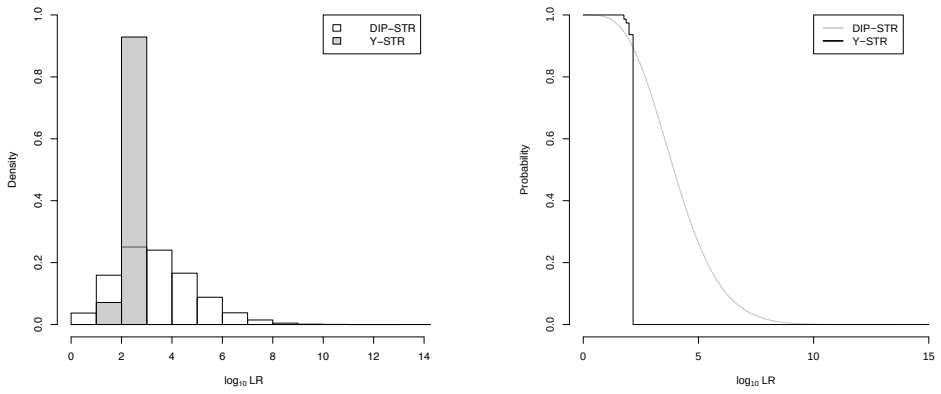
LR_d	Verbal equivalent	DIP-STR markers	STR markers
0	Exclusion	99.988	100
1-10	Limited support	0	0
10-100	Moderate support	0	0
100-1000	Moderately strong support	0.003	0
1000-10,000	Strong support	0.007	0
$> 10,000$	Very strong support	0.002	0

Figure 4.4: Percentage of DIP-STR and STR likelihood ratio values found for various intervals of probative strength for the hypothesis H_d.

From the defence's point of view the more the number of zeros among the simulated likelihood ratios, the more the method is desirable. Hence, Table 4.4 indicates that from the defence's point of view there is an advantage in using STR markers (for balanced mixtures), because the proportion of likelihood ratio values with 0 is maximal, while using DIP-STR markers 0.012% of simulated cases offer a false positive. In principle, the same considerations outlined in Section 4.4.1, which ascribe the difference in the overall likelihood ratio distribution to the different number of markers in the two kits, can be made in the case here. But even if one chooses the 9 STR markers which have the highest number of non-zero values (D8, D3, D1S, D12, VWA, D2S1, D18, FGA, D2S4) and then multiply them to obtain the overall likelihood ratio, one comes to the same conclusion, since 100% of 0 likelihood ratio values are obtained.

4.4.3 Comparison between DIP-STR and Y-STR marker systems assuming the point of view of the prosecution

Before proceeding with the details of the comparison between DIP-STR and Y-STR, it is useful to recall that this comparison is meaningful only under assumption **A6 for Y-STR**, that is when the known contributor is female and the unknown is a male. No assumption is needed, however, about the mixture proportion. As in Sections 4.4.1 and 4.4.2, the two likelihood ratio distributions to be compared are represented in terms of superimposed histograms and Tippett plots in Figure 4.5.



(a) (b)

Figure 4.5: Comparisons of the $\log_{10}\mathbf{LR_p^{Y-STR}}$ and $\log_{10}\mathbf{LR_p^{DIP}}$ distributions using superimposed histograms (a) and Tippett plots (b).

Since the histogram for the distribution of Y-STR likelihood ratio is composed by only two bars, Table 4.5 is retained here as a tabular summary.

Table 4.5 and Figure 4.5 indicate that, from the point of view of the prosecution, the use of DIP-STR markers appears more useful than that of Y-STR markers. With the latter, one can obtain at best a moderately strong support, while with DIP-STR markers an equal or

LR_p	Verbal equivalent	DIP-STR markers	Y-STR markers
0	Exclusion	0	0
$1-10$	Limited support	3.708	0
$10-100$	Moderate support	15.939	7.123
$100-1000$	Moderately strong support	25.037	92.877
$1000-10,000$	Strong support	24.026	0
$>10,000$	Very strong support	31.288	0

Table 4.5: Percentage of $\mathbf{LR_p^{DIP}}$ and $\mathbf{LR_p^{Y-STR}}$ values which fall into different categories of probative strength for H_p

higher degree of support is obtained in more than 80% of the cases, while a lower degree of support is obtained only in less than the 4% of the cases.

4.4.4 Comparison between DIP-STR and Y-STR marker systems assuming the point of view of the defence

Table 4.6 summarises the percentage of likelihood ratio values that fall into different categories of probative value, for simulations performed according to the viewpoint of the defence.

LR_d	Verbal equivalent	DIP-STR markers	Y-STR markers
0	Exclusion	99.988	99.31
$1-10$	Limited support	0	0
$10-100$	Moderate support	0	0.098
$100-1000$	Moderately strong support	0.003	0.592
$1000-10,000$	Strong support	0.007	0
$>10,000$	Very strong support	0.002	0

Table 4.6: Percentage of $\mathbf{LR_d^{DIP}}$ and $\mathbf{LR_d^{Y-STR}}$ values that fall into different categories of probative strength for H_p.

This table indicates that a comparison between DIP-STR and Y-STR markers (from the point of view of the defence), should take into consideration two factors. First, if one seeks to be conservative about the number of times in which a likelihood ratio greater than zero is obtained, the use of DIP-STR markers appears slightly preferable. Second, if one seeks to be conservative with respect to the strength of support obtained for values which are greater than zero, then Y-STR markers should be preferred, since – at worst – only a moderate support is obtained for those cases. Stated otherwise, one can consider two main options. One, the DIP-STR method, involves a higher number of zero likelihood ratio values, but with some possibility of a high likelihood ratio against a suspect who has a genotype 'compatible' with a mixture to which he is *not* a contributor. The other, the Y-STR method, involves a higher rate of likelihood ratios that would wrongly associate a suspect with a mixture. However, the likelihood ratios for such cases would be more moderate than in case of the DIP-STR method.

4.4.5 A discussion about the influence of genetic model assumptions

The problem of estimating Y-STR haplotype proportions is a fundamental one Brenner (2010). As already explained in Section 4.3.3, a Bayesian method is retained here for overall consistency with respect to what has been done for the STR and the DIP-STR methods. It is worth to emphasize, however, that the choice of a different method can lead to different simulation results and, consequently, to different conclusions about the comparison between the DIP-STR and the Y-STR methods. Among the alternative methods, the k-method Brenner (2010) and the discrete Laplace method Andersen et al. (2013b) have been chosen to investigate how substantial the difference in the conclusions would be. The k-method leads to essentially the same conclusions as those described above, both for the prosecution's and the defence's point of view. The choice of the discrete Laplace method results in a substantially different distribution for $\mathbf{LR_p^{Y-STR}}$, which would make the Y-STR method preferable from the prosecution's point of view. From the defence's point of view, the use of this method makes the DIP-STR and the Y-STR methods almost equivalent. This points out that there is a strong dependency on population genetic model assumptions. It is worthy of emphasis that there are inherent limitations in the state of the art, and whatever method is applied, its strengths and limitations should be carefully considered.

4.5 Consideration on the usefulness of the three methods

This section pursues a discussion on the proportion of cases in which each of the three methods cannot be used and therefore gives useful input to decision makers on their choice of the analytical methodology.

With regards to the STR method, it has already been explained that, in case of extremely unbalanced mixtures, this method is generally not useful to detect the minor contributor (see Section 4.1). In current practice, many or most extremely unbalanced mixtures probably go undetected, so that it appears difficult to assess the proportion of cases in which such mixtures are encountered.

In turn, it is easier to circumscribe the proportion of cases in which Y-STR markers are not useable. As noted in Section 4.2.3, that is the case whenever the major and the minor contributors are not a female and a male, respectively.

With regards to DIP-STR markers, there is only one situation in which this marker system is not useful. That is, when for all nine DIP-STR markers the major contributor is DIP-heterozygous (see also Table 4.1). In fact, as explained in Section 4.2.1, in case the known contributor is homozygous for the DIP allele, the fact of obtaining no alleles for the second contributor gives information about the DIP alleles of the minor contributor. The proportion of such kind of cases in the population can be assessed using the estimated allele proportions of each marker. This result is displayed in Table 4.7, which provides, for each marker, the probability that an individual (taken here as the major contributor) is heterozygous, or that both contributors are homozygous for same DIP allele (S or L), within the corresponding

likelihood ratio. Actually, these are cases in which the likelihood ratio has the lowest values, independently on the STR parts which constitute the DIP-STR minor contributor's genotype. The last column in the table gives the probability that in all markers the major contributor is DIP-heterozygous, or that both contributors are homozygous for the same DIP alleles (S or L).

	Marker 1		Marker 2		Marker 3	
	LR	Probability	LR	Probability	LR	Probability
Major heterozygous	1	0.374	1	0.475	1	0.442
Both homozygous S	1.772	0.318	2.678	0.139	9.17	0.012
Both homozygous L	16.146	0.004	6.612	0.023	2.229	0.201

	Marker 4		Marker 5		Marker 6	
	LR	Probability	LR	Probability	LR	Probability
Major heterozygous	1	0.335	1	0.475	1	0.342
Both homozygous S	1.613	0.384	2.678	0.139	20.816	0.002
Both homozygous L	22.11	0.002	6.612	0.0229	1.640	0.372

	Marker 7		Marker 8		Marker 9	
	LR	Probability	LR	Probability	LR	Probability
Major heterozygous	1	0.457	1	0.403	1	0.077
Both homozygous S	2.392	0.175	1.931	0.268	1.086	0.848
Both homozygous L	8.003	0.016	12.721	0.006	613.938	2.65×10^{-6}

	All markers	
	LR	Probability
Major heterozygous	1	6.12×10^{-5}
Both homozygous S	19631.581	2.59×10^{-9}
Both homozygous L	$3{,}57\times10^{9}$	7.86×10^{-20}

Table 4.7: The probability of occurrence of the 3 lowest likelihood ratio values obtained with DIP-STR markers, namely the values corresponding to cases in which the major contributor is heterozygous, or both contributors are homozygous for the same DIP allele.

It is worth noting that probabilities in Table 4.7 are not derived from the simulations of mixtures. They are calculated on the basis of the allele proportions relating to the databases of interest. The probability of a genotype that in all markers is heterozygous for the DIP allele is 6.12×10^{-5} (see last column of Table 4.7). This means that, on average, in only about 0.00612% of the cases a mixture, analysed with DIP-STR markers, does not help in discriminating between the two hypotheses of interest. This proportion seems remarkably small. In an actual case, it may thus be of interest to compare this proportion with the probability of facing an unbalanced mixture that may not lead to appropriate results with the traditional STR technique (to be assessed in the light of the case circumstances). However, this argumentation takes as an assumption that the mixture has already been recognised as such. In fact, there are situations (typically the case in which the two contributors are DIP-homozygous of the same type) in which a LR $\neq 1$ is obtained (as already pointed out in Section 4.2.1, only if one presumes the presence of a second contributor and the genotype of a suspect is also available.

Contrary to what happens with the use of STR markers and Y-STR markers, where often the second contributor to the mixture is missed without even suspecting his (her) presence,[2] with the use of DIP-STR markers it is sometimes possible to know with certainty and in advance the impossibility of detecting the genotype of the second contributor (i.e., when the major contributor is DIP-heterozygous in all markers). In general, using DIP-STR markers a mixture cannot be recognised as such when in all markers either the major contributor is heterozygous or both contributors are DIP-homozygous of the same type. The probability that an actual two-person mixture will not be recognised as such (i.e., the presence of a second contributor cannot be pointed out) has been calculated, using the allelic proportions, as the probability that in each marker either the major contributor is DIP-heterozygous or the two contributors are DIP homozygous of the same type. This probability is equal to 0.039. This means that about 4% of recovered stains, which are actually mixtures, will leave one with uncertainty about the presence of a second contributor.

4.6 Conclusion

The research reported in this article aimed at comparing three profiling methods for analysing DNA mixtures of two contributors. The relative advantages and limitations of STR markers, DIP-STR markers and Y-STR markers was considered from the point of view of the defence and the prosecution. In such a comparison, different aspects appear relevant, such as the proportion of cases in which mixtures have characteristics that make a given method useful (see, e.g., Section 4.5), and the distribution of likelihood ratio results in scenarios that reflect the viewpoint of either the prosecution or the defence (i.e., propositions of interest H_p and H_d, as defined in Section 4.3).

For cases of, at worst, moderately unbalanced mixtures, the simulation results − that is the distributions of the likelihood ratio values both from the prosecution's and the defence's point of view − suggest that the traditional STR marker system should be preferred. The case is different for extremely unbalanced mixtures. Here, STR markers are not reliable, but Y-STR markers and DIP-STR markers are applicable (Section 4.5). In such cases, the latter method should be preferred from the prosecution's point of view, since in about the 80% of the cases one obtains likelihood ratios which are higher than those obtained with the Y-STR method. However, from the the defence's point of view, two aspects should be reminded: one aspect concerns the strength of support obtained in case of a wrong association (i.e., when the likelihood ratio supports the wrong proposition), the other aspect relates to the number of times in which such a wrong indication is encountered. This is why from the defence's point of view, preferences may depend on what aspect one considers.

The common way to detect the presence of a possible second contributor to a stain already typed for STR markers (and which appeared as a single mixture), is to use Y-STR markers. However, this approach too, can miss the minor contributor if the gender composition of the two contributors is not proper (i.e., the major one is female and the minor one is male). The

[2]With the use of quantification methods it is possible to detect the presence of a second contributor, but only for the good gender mismatch between the two contributors: the major one should be female and the minor one should be male.

use of DIP-STR markers can thus be desirable for all those kind of traces that, with the use of STR and Y-STR markers, appear as single stain, but for which one suspects the presence of a second contributor. In these cases, DIP-STR markers can also complement Y-STR results to discriminate paternally related individuals.

Actually, the use of DIP-STR markers could present an interest for all kind of DNA stains, independently of the use of STR markers. The reason for this is that with the use of DIP-STR markers one can establish in advance if this method could be used, because it starts by determining the genotype of the assumed known major contributor (see Section 4.5). In case of a favourable outset, DIP-STR profiling can provide information about the second contributor in terms of one, two or no alleles (Section 4.2.1). Although the likelihood ratio distributions obtained under the defence's and the prosecution's point of view are not as marked as those that can be obtained with traditional STR markers, they can still be regarded as practically useful (see, e.g., Tables 4.10 and 4.11). In addition, new DIP-STR markers are currently investigated. This may favourably improve the likelihood ratio distributions that could be obtained under the various competing points of view in a near future, but analysts should also remind that the definition of practical procedures will also encompass additional factors such as time and monetary constraints.

Appendix A. Additional tables and figures

Marker name	Min	1st Quantile	Median	Mean	3rd Quantile	Max
VWA	0.54	0.74	0.97	1.04	1.27	2.36
TH01	0.45	0.62	0.8	0.86	0.99	2.57
SE33	1.48	2.04	2.29	2.38	2.65	4.37
FGA	0.78	0.98	1.269	1.35	1.67	4.33
D22	0.37	0.46	0.68	0.86	0.99	4.32
D21	0.59	0.85	1.13	1.27	1.5	4.33
D19	0.42	0.62	0.94	1.14	1.54	4.33
D18	0.89	1.13	1.39	1.48	1.69	3.93
D16	0.43	0.58	0.78	0.92	1.1	3.77
D12	0.98	1.35	1.63	1.71	2.06	4.34
D10	0.43	0.58	0.76	0.89	1.08	3.09
D8	0.51	0.77	1.07	1.14	1.37	4.32
D3	0.56	0.66	0.80	0.87	1.03	2.70
D2S4	0.34	0.47	0.77	0.94	1.35	4.15
D2S1	0.78	1.18	1.43	1.50	1.73	4.33
D1S	0.97	1.36	1.58	1.63	1.85	4.16
All markers	13.78	18.66	19.9	20	21.22	29.85

Table 4.8: The summaries of the distributions of the $\log_{10}$ of the likelihood ratio values for each STR marker and for the overall $\log_{10}\mathbf{LR}_{\mathbf{p}}^{\mathbf{STR}}$ (last row).

LR	Verbal equivalent	VWA	TH01	SE33	FGA	D22	D21
0	Exclusion	92.191	89.122	99.464	95.572	85.816	93.698
$1-10$	Limited support	5.818	9.694	0	1.889	13.415	4.377
$10-100$	Moderate support	1.981	1.175	0.221	2.513	0.749	1.852
$100-1000$	Moderately strong support	0.01	0.009	0.306	0.025	0.02	0.07
$1000-10,000$	Strong support	0	0	0.009	0.001	0	0.003
$>10,000$	Very strong support	0	0	0	0	0	0

LR	Verbal equivalent	D19	D18	D16	D12	D10	D8
0	Exclusion	90.169	96.695	88.581	97.85	88.229	92.437
$1-10$	Limited support	8.593	0.606	9.478	0.05	10.659	5.41
$10-100$	Moderate support	1.181	2.645	1.904	1.963	1.102	2.123
$100-1000$	Moderately strong support	0.057	0.053	0.021	0.135	0.008	0.029
$1000-10,000$	Strong support	0	0.001	0	0.002	0.002	0.001
$>10,000$	Very strong support	0	0	0	0	0	0

LR	Verbal equivalent	D3	D2S4	D2S1	D1S	All markers
0	Exclusion	90.102	86.229	96.701	97.682	100
1-10	Limited support	8.116	12.735	0.937	0.023	0
10-100	Moderate support	1.775	1.01	2.293	2.244	0
100-1000	Moderately strong support	0.007	0.026	0.068	0.051	0
1000-10,000	Strong suppor	t0	0	0.001	0	0
$>10,000$	Very strong support	0	0	0	0	0

Table 4.9: Percentage of likelihood ratio values belonging to the different intervals of probative value in favour of the proposition H_p, for each STR marker and combined across all markers (last column). Marker names are abbreviated to their first three characters.

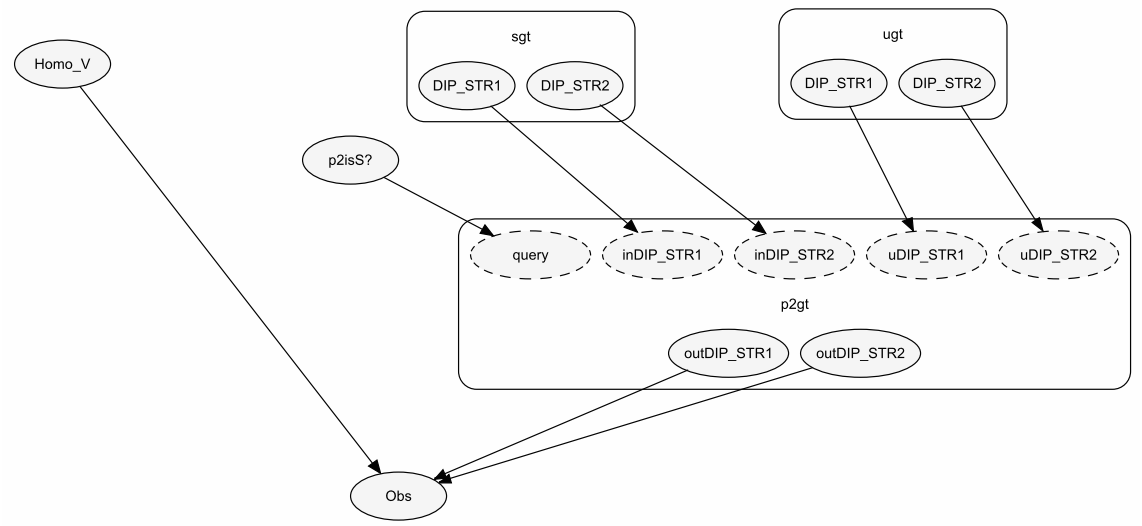


Figure 4.6: Object-oriented Bayesian network for evaluating DIP-STR profiling results of mixtures from two contributors, when DIP-STR markers are used (Cereda et al., 2014b).

Marker name	Min	1st Quantile	Median	Mean	3rd Quantile	Max
Marker 1	0	0	0.25	0.46	0.94	3.79
Marker 2	0	0	0.43	0.41	0.60	3.77
Marker 3	0	0	0.35	0.41	0.52	3.76
Marker 4	0	0	0.21	0.41	0.90	3.61
Marker 5	0	0	0.43	0.44	0.71	3.59
Marker 6	0	0	0.21	0.35	0.78	3.78
Marker 7	0	0	0.24	0.37	0.63	3.46
Marker 8	0	0	0.29	0.40	0.74	3.58
Marker 9	0	0.04	0.04	0.11	0.04	3.62
All markers	0	2.23	3.20	3.37	4.32	13.71

Table 4.10: The summaries of the distributions of the $\log_{10}$ of the likelihood ratio values for each DIP-STR marker and for the overall $\log_{10}\mathbf{LR}_{\mathbf{p}}^{\mathbf{DIP}}$ (last row).

LR	Verbal equivalent	Marker 1	Marker 2	Marker 3	Marker 4	Marker 5
0	Exclusion	63.353	77.401	70.57	58.877	79.069
$1-10$	Limited support	35.317	21.579	28.179	40.314	19.924
$10-100$	Moderate support	1.312	0.997	1.244	0.755	0.997
$100-1000$	Moderately strong support	0.017	0.023	0.007	0.054	0.01
$1000-10,000$	Strong support	0.001	0	0	0	0
$>10,000$	Very strong support	0	0	0	0	0

LR	Verbal equivalent	Marker 6	Marker 7	Marker 8	Marker 9	All markers
0	Exclusion	58.486	69.142	67.975	14.802	99.988
$1-10$	Limited support	41.272	29.543	31.262	84.548	0
$10-100$	Moderate support	0.239	1.223	0.753	0.65	0
$100-1000$	Moderately strong support	0.003	0.092	0.01	0	0.003
$1000-10,000$	Strong support	0	0	0	0	0.007
$>10,000$	Very strong support	0	0	0	0	0.002

Table 4.11: Percentage of likelihood ratio values obtained for the various categories of probative value in favour of H_p, for each marker and combined across all markers, when the defence's point of view is considered.