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A new methodological framework to interpret complex DNA profiles using likelihood ratios

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ABSTRACT

Although likelihood ratio (LR) based methods to analyse complex mixtures of two or more individuals, that exhibit the twin phenomena of *drop-out* and *drop-in* has been in the public domain for more than a decade, progress towards widespread implementation in to casework has been slow. The aim of this paper is to establish a LR-based framework using principles of the *basic model* recommended by the ISFG DNA commission. We use the tools in the form of open-source software (LRmix) in the Forensim package for the R software. A generalised set of guidelines has been prepared that can be used to evaluate any complex mixture. In addition, a validation framework has been proposed in order to evaluate LRs that are generated on a case-specific basis. This process is facilitated by replacing the reference profile of interest (typically the suspect's profile) with simulated random man using Monte-Carlo simulations and comparing the resulting distributions with the estimated LR. Validation is best carried out by comparison with a standard. Because LRmix is open-source we proposed that it is ideally positioned to be adopted as a standard basic model for complex DNA profile tests. This should not be confused with 'the best model' since it is clear that improvements could be made over time. Nevertheless, it is highly desirable to have a methodology in place that can show whether an improvement has been achieved should additional parameters, such as allele peak heights, are incorporated into the model. To facilitate comparative studies, we provide all of the necessary data for three test examples, presented as standard tests that can be utilised to carry out comparative studies. We envisage that the resource of standard test examples will be expanded over coming years so that a range of different case-types that are included will be used in order to improve the efficacy of models; to understand their advantages; conversely, to understand any limitations and to provide training material.

1. Introduction

In this paper we illustrate the application of exploratory data analysis using likelihood ratios (theory outlined by Haned et al. [1]), applied to the interpretation of complex DNA profiles. An important principle of the methodology is that the incorporation of the twin effects of *drop-out* and *drop-in* [2] into the interpretation strategy [3] enables a meaningful comparison to be made between any crime-stain and any reference sample. This is possible because there is no longer any requirement to think in terms of 'match' or 'non-match' [4]. A traditional analysis is a two-step consecutive process: a) Is there a match? b) What is the strength of evidence if there is a match? A numeric strength of evidence is usually formulated to support a prosecution hypothesis ($LR > 1$) and this is a weakness of the traditional approach. However, by using a suitable model that by-passes the requirement to decide a definitive 'match' based on subjective criteria, there is no reason why the strength of evidence cannot also be calculated in favour of the defence hypothesis ($LR < 1$). Subjective assessments of cases are therefore avoided because the statistical model employed [5,6] is able to simultaneously measure strength of evidence that could favour the defence hypothesis, as well as the prosecution hypothesis.

In the past, mixture interpretation has been difficult to standardise. Different laboratories follow different mixture interpretation guidelines [7,8] and the diversity of casework and associated propositions encountered renders the generalisations of such guidelines difficult. It is therefore desirable to develop an interpretation framework that not only facilitates associating a weight to any type of DNA evidence, but also provides a way of testing the reliability of the obtained results. Haned et al. [1] have developed an exploratory approach, anchored in a likelihood ratio framework, which addresses these two requirements. Relying on

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their approach, the key features of the proposed framework are summarised as follows:

a) There is no requirement to make an assessment about *whether* an analysis should be carried out based on subjective criteria to decide a 'match' or 'non-match' with a reference sample. There is no inherent restriction on comparing any crime stain(s) with any reference sample.
b) Because the analysis of crime and reference samples is concurrent, the Clayton recommendation [9] that consecutive examination of reference samples at the end of the interpretation process, is no longer a requirement.
c) There is no need to filter any of the allelic peaks within our framework – apart from selecting all alleles that are above the limit of detection threshold (LDT). If ambiguous allelic peaks are present (stutters) then they are incorporated directly into the analysis.
d) The shift of focus is firmly towards the formulation of propositions (hypotheses provided by the defence and prosecution). Because propositions can only be described in pairs, it may not be obvious which to consider, especially with low-template DNA analysis with no identifiable body fluid. To facilitate, we provide guidance to estimate the minimum number of contributors.
e) The interpretation process is regarded as exploratory since the results can be conditioned on different circumstances that are considered to be relevant to the case.
f) Performance testing is built into the interpretation process and is used to *evaluate* reported likelihood ratios; acting as a component of *validation* (we discuss this concept in detail in Section 9.1). It is proposed that the LRmix module fulfils the requirements to act as a *standard*. Consequently, it can be used to determine whether alternatives (or changes to existing models) result in improved performance.

To illustrate the principles, we evaluate three different cases in order to explore the effect of conditioning, and to demonstrate the importance of simplifying the hypotheses used to describe the circumstances of a case. These cases are also submitted as a set of standard test examples (see electronic supplement) that can be utilised as a resource for others to carry out comparative tests with other models, or model improvements, thereby fulfilling an important requirement for validation exercises.

2. Characterisation of low template DNA profiles

There has been recent debate about the characterisation of a low-template DNA, but the position is now summarised by a recent ISFG DNA commission [10].

LT-DNA profiles usually exhibit some degree of allele *drop-out*. DNA profiles can be characterised and classified as LT-DNA vs. standard DNA profiles by comparison of peak heights to a stochastic threshold (T), determined by logistic regression [11,12]. The threshold measures the *risk* of allelic dropout if allele peak heights are between the lower limit of detection threshold (LDT) and the stochastic threshold (T). In addition, allele *drop-in* may be observed – its frequency tends to increase with higher sensitivity of detection (e.g. elevated cycle number; increased injection time). Degradation can also affect a DNA profile so that it appears standard at low molecular weight loci, and low template at high molecular weight loci [13]. Alternatively, *differential degradation* may occur where the relative amounts of degradation vary per contributor and this in turn affects the mixture proportion (Mx) [14,15] across the DNA profile.

If all contributors are within the low template range then the relationship between the DNA quantity allelic peak heights tends towards a uniform distribution, so that heterozygotes become increasingly unbalanced. These stochastic effects are predictable however, and computer simulations [16,17] have demonstrated a sound theoretical basis to explain heterozygote balance and allele drop-out relative to DNA quantity.

2.1. Analysis of LT-DNA profiles

Historically, LT-DNA profiles were first interpreted using the consensus profile method [2] where only alleles observed in two or more replicate profiles were reported; a variation of the method was described by Benschop et al. [18–20]. In addition, composite profiles are sometimes reported: here profiles are combined in their entirety to form a single combined genotype – the robustness of this strategy was investigated by Bright et al. [17]. Consequently, several methods are in current use to interpret LT-DNA profiles. None is ideal because not all of the information in the DNA profile is utilised. It is not possible to incorporate the allele *drop-out* and *drop-in* phenomena in probabilistic terms. This may lead to anti-conservativeness [21] and readers are referred to the ISFG DNA commission paper [10] for further clarification of the arguments. Therefore, there are strong reasons in favour of the introduction of 'new' probabilistic approaches, since all of the information in replicate profiles is analysed without the need to construct a consensus or composite profile [1,4]. The incorporation of *drop-in* and *drop-out* into the model fulfils the criteria suggested by the ISFG DNA commission [10], greatly reducing anti-conservative risks since LRs < 1 can be assigned to loci. Whereas the complexity of applying consensus and composite methods restricted their use to profiles categorised as non-mixtures and simple mixtures, probabilistic methods are not restricted by the number of replicates, or the number of contributors. This leads to the necessity to move the focus of the discussion to the formulation of propositions.

3. DNA profiling evidence, transfer and propositions

The interpretation of all DNA profiling evidence has to be considered in the context of the case-circumstances. Increased sensitivity of detection quite often means that there is no body fluid or cell type that can be associated with the DNA profile if the profiling evidence has been recovered from a touched surface. When this occurs it seems to be common practice to attribute the profile to epithelial cells (but there is usually no direct evidence for this assumption). It is never implicit that the recovery of a DNA profile is associated with a crime-event [22], and alternative methods of DNA transfer must always be a consideration when hypotheses are formulated, especially when LT-DNA is analysed.

It can be generalised that contributors to a DNA profile will always comprise *known* individuals (victim(s), suspect(s) and witnesses) and zero or more *unknown* individual(s). When a profile consists of DNA from several contributors, it cannot be assumed that each was deposited concurrently on a surface (e.g. weapon). It is inevitable that depositions will be made before, during and/or after a crime event – the reader is referred to [23] for an outline of these principles.

The number of contributors is itself often uncertain – unknown individuals are more common in LT-DNA profiles and the 'masking effect', where alleles are shared between different contributors [24], complicates the assessment. The more contributors there are, the more likely it is that the total will be underestimated. Maximum likelihood principles [19,25,26], can assist reporting officers in deriving the most plausible number of contributors that can explain the observed epgs. Additional tests, such as Y-chromosome analysis, are often useful to determine the number of male contributors. Therefore the elucidation of the absolute