



Complex DNA mixture analysis: Report of two cases



Giorgia Tasselli^a, Massimo Lancia^{a,*}, Federica Tommolini^a, Gabriele Margiotta^b,
Susanna Massetti^a, Eugenia Carnevali^a

^a University of Perugia, Section of Legal Medicine, Italy

^b University of Siena, Section of Legal Medicine, Italy

ARTICLE INFO

Article history:

Received 27 August 2015

Received in revised form 14 September 2015

Accepted 9 October 2015

Available online 27 October 2015

ABSTRACT

During the last years, DNA profiling techniques have become more sensitive and mixtures with multiple contributors occur often. Nevertheless, interpretation of mixture profiles is the most complex task in the forensic field. The ISFG published recommendations for the interpretation of mixed DNA profiles that may have allelic drop-out or drop-in. The DNA commission of the ISFG has supported the use of likelihood ratios (LRs) as preferable to other methods. LR can be calculated by probabilistic programs, using semi and fully continuous (probabilistic) methods. These methods increase the efficiency of forensic laboratories and improve the consistency and transparency of the reported results. LRmix is an open-source system dedicated to the interpretation of forensic DNA profiles with a particular focus on complex DNA mixtures. This software is programmed on a LR model and facilitates the calculation of LR for complex mixtures, with partial profiles with known and unknown contributors. Here, we report the re-analysis of two old complex cases processed in our laboratory in the last years. In both cases, we used the software LRmix Studio with interesting consequences: in one case we solved the crime, in the other one, the results allowed the case to be reopened.

© 2015 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

In recent years, improvements in DNA typing technologies have fueled the analysis of increasingly complex profiles in forensic caseworks. A 'complex DNA profile' is defined as a genetic profile that is subject to allele drop-out and/or allele drop-in. Also mixtures can be grouped within this definition [1]. Thanks to more sensitive DNA profiling techniques, low level complex DNA mixtures of three or more contributors become more and more widespread in casework, and their interpretation more challenging [2]. In the past, different laboratories followed different mixture interpretation guidelines, demonstrating the need for standardization [1]. For this reason, in 2012, the DNA commission of the ISFG encouraged more research and the creation of a statistical software to help the development and implementation of universally accepted standards [3]. The DNA commission of the ISFG has encouraged the use of likelihood ratios (LRs), that is considered preferable compared to other methods [4,5]. The LR is considered to be the most powerful and relevant measure of the weight of evidence. But the lack of accessible software to implement

appropriate methods to interpret forensic samples, has delayed the introduction of LR-based methods. Luckily, in recent years significant progress has been made; new computer programs that can use different probabilistic models have been introduced [2]. A number of different LR-based models have been described, and they are traditionally classified into two categories: the so-called *continuous models* incorporate peak heights as continuous variables, and therefore account for both the qualitative and quantitative data provided by the electropherograms and the *semi-continuous or qualitative models* is based on the list of alleles observed in the electropherogram. In general qualitative models are easier to implement as they rely on fewer assumptions [1] and among available programs, LRmix is one of the most widespread in forensic laboratories. LRmix is an open-source system dedicated to the interpretation of forensic DNA profiles, with a particular focus on complex DNA mixtures. December 15th 2014 saw the first release of LRmix Studio [6]. This software, like LRmix, is programmed on the likelihood ratio model described by Haned [2] and Gill [1], and facilitates the calculation of likelihood ratios for complex mixtures, including partial profiles and with known and unknown contributors. Here, we report the re-analysis of two old complex cases processed in our laboratory in the last years. In both cases, we used the open-source software LRmix Studio with interesting consequences: in fact, in one case, the results allowed the case to be reopened, and in the other one, we solved the crime.

* Corresponding author at: Section of Legal Medicine, University of Perugia, Piazza Severi 1 Perugia, Italy. Fax: +39 0744220236.

E-mail address: dr.massimolancia@gmail.com (M. Lancia).



Fig. 1. Coat (case 1) and old boot (case 2).

2. Materials and methods

We report the re-analysis of two old complex cases processed in our laboratory in the last years. Here, we briefly describe the two cases.

2.1. Case no. 1

In 2012, a young, Caucasian boy died hit by a train. Investigators believed it was a suicide, but the victim's family thought he was murdered. Relatives of the victim submitted the relatively new coat (Fig. 1b) that the boy was wearing at the time of the accident, in order to detect traces of genetic material belonging to persons other than the victim. Investigation of the coat revealed a mixed profile under the armpit of the coat, as if someone had picked up and pushed the victim under the train. The profile was analysed with LRmix Studio and suggested the intervention of several people in causing the victim's death. The family of the boy also gave the laboratory two cigarette butts smoked by two friends of the boy that were thought to be involved in the death of their relative. Finally, the case was re-opened based on these results.

2.2. Case no. 2

In 2012, a man was declared missing while he was walking on a mountain in a region in the middle Italy. After one year, the case was closed, although the body had not been found. Some months after, an old boot (Fig. 1a) was found near the mountain where the man had been seen for the last time. Investigation of the boot revealed a mixed profile that was analysed with LRmix Studio. The results suggested that the boot could belong to the missing man. The case was reopened, the surrounding area from where the boot was found was searched again, and the body of the missing person was recovered.

3. Discussion

Undoubtedly, interpretation of mixtures of DNA profiles has proven to be the most complex task in the forensic field. The ISFG-DNA commission suggested LR to be the preferred way to associate strength of evidence of DNA mixtures [7]. Classical LR approaches used a binary model (based on exclusion/inclusion criteria) that forced decisions to be made about reporting peak signals near

stochastic thresholds, because the numerator only could take 0 or 1 values [7]. Luckily, in the last years, newly developed LR models that account for PCR-related stochastic effects (allelic drop-out, drop-in, stutters), have enabled the analysis of complex cases, otherwise reported as inconclusive [2]. These new semi-continuous LR models allowed an increase in the number of complex cases that can be evaluated and reported to the court. On the other hand, LR formulation becomes more complex and for this reason the use of software is mandatory to carry out these types of evaluations. Assumptions about the numbers of contributors follow an examination of the electropherograms. These assumptions may be different between the prosecution and the defense hypotheses. After this initial evaluation, it is therefore necessary to evaluate several sets of hypotheses for any case. Once the hypotheses are formulated, the mathematical analysis is complex and its evaluation can be made only by the use of specialist software. It is essential that forensic analysts be trained, not only in the use of the software, but also in understanding likelihood ratio concepts [7]. For these reasons, it is mandatory for every forensic laboratory that there is a specialist operator who is expressly trained for evaluation of these LR models and for use of this software. Furthermore, it is desirable that this software becomes more and more user-friendly, so that their use becomes more widespread in every forensic laboratory.

4. Conclusions

LRmix Studio is a Java-based, open access software, programmed on the likelihood ratio model described by Haned [2] and Gill [1], that facilitates the calculation of LR for complex mixtures. As demonstrated with the above case examples this is a very useful tool to deal with complex scenarios. A great advantage of this program is that the calculations of strength of evidence are hugely simplified. The removal of subjectivity obtained by using this program means that a greater consistency of reports should be achieved [7].

References

- [1] P. Gill, et al., A new methodological framework to interpret complex DNA profiles using likelihood ratios, *Forensic Sci. Int. Genet.* 7 (February (7)) (2013) 251–263.
- [2] H. Haned, et al., Complex DNA mixture analysis in a forensic context: evaluating the probative value using a likelihood ratio model, *Forensic Sci. Int. Genet.* 16C (November) (2014) 17–25.

- [3] J.A. Bright, et al., A series of recommended tests when validating probabilistic DNA profile interpretation software, *Forensic Sci. Int. Genet.* 14 (January) (2015) 125–131.
- [4] P. Gill, et al., DNA commission of the ISFG: recommendations on the interpretation of mixtures, *Forensic Sci. Int.* 160 (July (2–3)) (2006) 90–101.
- [5] P. Gill, et al., Interpretation of complex DNA profiles using empirical models and a method to measure their robustness, *Forensic Sci. Int. Genet.* 2 (March (2)) (2008) 91–103.
- [6] <http://lrmixstudio.org/download/>.
- [7] L. Prieto, et al., EuroforGen-NoE collaborative exercise on LRmix to demonstrate standardization of the interpretation of complex DNA profiles, *Forensic Sci. Int. Genet.* 9 (March) (2014) 47–54.