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Research paper

Stabbing simulations and DNA transfer

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ABSTRACT

Technical developments have made it possible to analyze very low amounts of DNA. This has many advantages, but the drawback of this technological progress is that interpretation of the results becomes increasingly complex: the number of mixed DNA profiles increased relatively to single source DNA profiles and stochastic effects in the DNA profile, such as drop-in and drop-out, are more frequently observed. Moreover, the relevance of low template DNA material regarding the activities alleged is not as straightforward as it was a few years ago, when for example large quantities of blood were recovered. The possibility of secondary and tertiary transfer is now becoming an issue.

The purpose of this research is twofold: first, to study the transfer of DNA from the handler and secondly, to observe if handlers would transfer DNA from persons closely connected to them. We chose to mimic cases where the offender would attack a person with a knife. As a first approach, we envisaged that the defense would not give an alternative explanation for the origin of the DNA.

In our transfer experiments (4 donors, 16 experiments each, 64 traces), 3% of the traces were single DNA profiles. Most of the time, the DNA profile of the person handling the knife was present as the major profile: in 83% of the traces the major contributor profile corresponded to the stabber's DNA profile (in single stains and mixtures). Mixture with no clear major/minor fraction (12%) were observed. 5% of the traces were considered of insufficient quality (more than 3 contributors, presence of a few minor peaks). In that case, we considered that the stabber's DNA was absent.

In our experiments, no traces allowed excluding the stabber, however it must be noted that precautions were taken to minimize background DNA as knives were cleaned before the experiments. DNA profiles of the stabber's colleagues were not observed.

We hope that this study will allow for a better understanding of the transfer mechanism and of how to assess and describe results given activity level propositions. In this preliminary research, we have focused on the transfer of DNA on the hand of the person. Besides, more research is needed to assign the probability of the results given an alternative activity proposed by the defense, for instance when the source of the DNA is not contested, but that the activities are.

1. Introduction

Technical developments now allow the analysis of traces of low template DNA so that an increasing number of invisible DNA traces are collected at crime scenes. These items are submitted to the laboratory without knowledge of the cellular origin and they can rarely be safely attributed to a biological substance such as blood, semen or saliva [1]. The increase in sensitivity of these technical developments brings on two challenges when assessing the value of the results. First, interpretation becomes more complex due to the presence of mixtures and stochastic effects such as drop-out and drop-in [2]. Second, the relevance of the trace pertaining to the activity at hand is not as straightforward as it could be for blood or semen recovered in a considerable quantity. Dealing with smaller quantities of DNA means secondary and tertiary transfer often have to be considered [3]. A further challenge concerns the absence of DNA, for example on a knife that was used in a stabbing: indeed, the absence of material (or absence of so-called 'matching' material) needs to be assessed [4].

The purpose of this project was thus to study the transfer of DNA from an individual's hand to a knife handle when simulating a knife attack. Knowing the possibility of DNA secondary transfer exists, we were interested not only in the transfer of the stabber's

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Fig. 1. Picture illustrating the type of knife used in this study.

DNA, but also in the transfer of any extraneous DNA present on his/her hand (e.g., DNA from colleagues encountered the day the experiments were performed).

The objectives were set as follows:

- Observe the results (type of mixtures, quality of DNA profiles) when searching for DNA on a knife handle that had been used for stabbing;
- See how these results could be assessed given activity level propositions [5] using probabilistic reasoning [6,7];
- Study whether DNA profiles 'matching' the stabber's colleagues (met during the day of the experiment) were observed on the knife handle.

2. Materials and methods

64 experiments were carried out. In order to mimic stabbing, four individuals (two males and two females) were asked to 'stab' a cardboard box with a knife. They all used their dominant hand (two persons were left handed and two right handed). Four identical knives were used, one for each volunteer. The knife handles were made of flexible plastic (see Fig. 1).

Two experiments took place on the same day: the first at 11 am and the second at 4 pm. Right afterwards, traces were collected using the double swab method. First a wet, then a dry, cotton swab was used for the collection of the cells. The protocol was repeated for 8 days. In order to limit background DNA (i.e., DNA present for unknown reasons), the knife was cleaned between each experiment (by using bleach, ethanol and leaving the items under UV light for 30 min). To verify the absence of background, a negative control was taken from the knife after cleaning and before the experiment. Results were all negative (no DNA profile). In addition, each stabber had his/her own knife. This ensured that any background would come from the stabber's hands and not the knife itself. The persons' hands were washed at 8 am.[1] Otherwise the persons' hands were not washed in any particular way and they carried out their normal activities in their office environment (i.e., having lunch or coffee with their colleagues, speaking with them etc.).

DNA was extracted, using the combination of two kits, QIAshredder and QIAAmp kit (Qiagen AG, Hombrechtikon, Switzerland) and concentrated to 50 μL with Microcon 30 spin columns (Millipore AG, Zug, Switzerland) [8]. It was then quantified with a 7500 Real Time PCR System (Life Technologies Europe B.V., Zug, Switzerland), using Investigator® Quantiplex HYres (Qiagen) kit following standard protocols. This kit makes it possible to quantify male DNA and total DNA in the same trace DNA extract. Using this kit may show the presence of a DNA profile hidden by a major profile, if both individuals have different sex, mainly when the major contributor is a woman and the minor one is a man. Moreover, DNA was amplified (three times for each DNA extract to have three replicates) at 30 cycles using NGM SElect (Applied Biosystem-Life Technologies) kit and a PCR system 9700 (Applied Biosystem) and finally analyzed with 3130xl Genetic Analyser ABI (Applied Biosystem) and GeneMapper®IDX Software (Applied Biosystem). For all these protocols, the manufacturer's instructions were followed.

Even for low DNA concentrations such as 1 pg/μL, the trace was analysed. The analytical threshold used was 30 RFU.

In order to use all the information available on the entire electropherogram, all replicates (with all loci) from the same DNA trace were analysed together with STRmix™ v2.3.05 software [9–12], a forensic software that can resolve mixed DNA profiles based on a continuous approach. This software takes into account the peak heights and statistically accounts for the possibility of degraded DNA and stochastic variation such as stutter, allelic drop-in and drop-out. It also provides information regarding the mixture proportion and the weight that is given to the possible genotypes of the contributors. The weight is used to express how well a proposed genotype explains the profile.

We used the following procedure to record our results:

First, the number of contributors was determined based on the number of the peaks detected at each locus, peak height balance information and how the experiments were planned (i.e., we expected one person's DNA at least). Because the number of contributors was unknown, if a mixture of at least n persons was determined, for STRmix™ the number of contributors was also set as n. For three of the profiles this number was not consistent with the traces according to the program. In those cases, the number of contributors was set as $n+1$. A profile was considered unsuitable for comparison if there were more than 3 contributors. In those cases, it was not possible to assign the value of these profiles.

Second, in case of the mixtures, if a contributor had a mixture proportion higher than 70%, then the trace was considered to have originated from a major and at least one minor contributor. In that case, for each locus, the profile of the major and minor contributor (s) was defined if the combination had an assigned weight higher than 0.7.

A profile of a given person was considered as fully represented if, for all loci, the proposed combination had a weight higher than 0.7. The profile was considered partial but of value if for at least 6 loci, the person's genotype appeared as a combination with a weight higher than 0.7

An example of this procedure is given in an Appendix A.

Finally, the major and the minor DNA profiles of the traces were compared with the DNA profiles of the stabbers and of the colleagues they had met during the day.

When there was no major/minor contributors as defined (for example because the major portion had a mixture proportion less than 70% or if there were less than 6 loci where a combination had a weight higher than 0.7), or when the profile of the major and minor contributor(s) was not defined (because the combination had an assigned weight higher than 0.7 at less than 6 loci), then the profile of the stabber and of the colleagues they had met during

[1] The authors did not want to potentially recover DNA from the persons living with the stabbers to protect their privacy and limit the number of analyses. The stabbers were therefore asked to clean their hands when arriving at work. This measure increases the chance that the extraneous DNA originated from persons met at work or from unknown source.