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Proportion of total DNA consistent with the known owner on different areas of law enforcement owned firearms

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ABSTRACT

If a DNA profile obtained from evidence is consistent with an individual, there are several hypotheses on the activity level that could be evaluated in relation to the evidence. Was the DNA deposited by the owner or routine handler of the firearm? Was the individual's DNA transferred via another means to the firearm? Gaining insights as to the quantities of DNA typically obtained from an owner of a firearm from different areas of the item may aid the examiner in more effectively evaluating the evidence. This study focused on deoxyribonucleic acid (DNA) collected from the trigger and trigger guard, the frame and slide, and the front and rear sight areas of 16 law enforcement-issued firearms. All samples that were suitable for comparison supported the DNA profile under the proposition if the owner of the firearm was a contributor to the DNA profile obtained from the sample. Additionally, 93% of the samples were assigned a likelihood ratio (LR) associated with a contributor that was estimated to account for greater than 70% of the DNA profile. Establishing data that can be used for evaluative reporting will enhance the DNA examiner's ability to better explain the evidence in a courtroom setting.

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DNA; firearms; persistence; activity level propositions; STRmix™; evaluative reporting

1. Introduction

Locard's Exchange Principle notes that with every contact we leave a trace behind, conversely we also take some traces with us, this is the exchange[1]. It is expected that the owner of an item will leave biological material behind on that item when they are in contact with said item. What else are they transferring? Are they taking away some of their own cellular material in the process? The more we understand the typical quantities of DNA deposited on an item of evidence, the better we can evaluate the results we obtain in relation to competing hypotheses.

Firearms are common types of evidence submitted to forensic DNA laboratories for DNA profiling. With over 1 billion firearms in the world it is no surprise that they are routinely submitted as evidence to forensic laboratories, particularly in the United States of America where there are more privately owned firearms than there are people[2,3]. There is limited research on DNA profiles obtained from firearms, and much research of late has

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been focused on fired cartridge cases[4–10]. Evaluative reporting is any type of reporting that includes evaluating statements[11]. In order to utilize evaluative reporting, data must exist that can be utilized for evaluating the results of the evidence examination given competing hypotheses. This study sought to fill a gap in the existing data as it pertains to the persistence of 'owner DNA' on a firearm. The vast majority of firearms fall in to one of four categories: law enforcement owned, military owned, legally owned by a civilian, illegally owned by a civilian. This research focused on law enforcement owned firearms to obtain data that may be useful in evaluating evidence in law enforcement involved shootings and recovered firearms stolen from law enforcement. Data in this study may also be useful in evaluating evidence obtained from firearms in the remaining categories, although further research would need to be conducted for comparison.

2. Methods

This study consisted of measuring the quantity of DNA recovered from firearms and subsequently comparing the DNA profiles obtained to that of the owner of the firearm using probabilistic genotyping. The firearms used in this study were law enforcement issued pistols. Legally possessed firearms have been selected for the purpose of safety, having only trained individuals present whilst handling the firearm. All personnel used for this study are each assigned a specific firearm from their employer, the firearms are not shared. Reference buccal swabs were collected from the associated firearm owners who were designated Donors A-P as were their respective firearms. For example, the donor designated A's firearm was also designated A. All donors were adults over the age of 21. Donors A-F, H-I, and M-N were males and donors G, J-L, and P were females. Buccal swab collection consisted of a cotton swab rubbed on the inside of the mouth of the donor.

The sampling method utilized for the firearms was a single wet (moistened with DNA-free water) cotton swab followed by a dry cotton swab in three target areas: the trigger and trigger guard area, the front and rear sights, and the frame and slide. These are three areas routinely used for sampling in casework. The frame and slide areas encompass the main body of the firearm, the front and rear sights are the notched areas along the top of the firearm, and the trigger and trigger guard can be found in the crook of the gun with the guard surrounding the trigger. All swabs used for reference and firearm collection were Puritan brand cotton swabs.

Samples were prepared by cutting the swab material from the swab shaft with a single-use disposable sterile scalpel and transferring the swab cuttings into extraction tubes. Samples were processed using a manual extraction method. Each sample was lysed with 400 microlitres (µL) of digest buffer (1 M Tris-HCl, 0.5 M EDTA, 20% Sodium Dodecyl Sulfate), 30 µL of 10 mg/mL Proteinase K, and 20 µL of 1.0 M DTT with an overnight incubation at 56°C. The samples were vortexed, centrifuged, and substrate removed via spin basket centrifugation. Subsequently, a phenol/chloroform extraction was employed. The final elution volume was 30 µL.

All samples were quantified using the Promega PowerQuant® system on the Applied Biosystems 7500 Platform and amplified using Applied Biosystems GlobalFiler or the Promega PowerPlex® DNA profiling Fusion 6C systems on the ProFlex™ PCR System Thermal Cycler. Note, two amplification systems were selected based on reagent availability, and to reflect the data typically obtained in casework at the research laboratory.

Quantitation, normalization, and amplification were automated on a TECAN Freedom EVO 150 system. Normalization was optimized for a 1.0 nanogram (ng) optimal input, regardless of system, following the laboratory's existing standard operating procedures (SOPs). Both systems have comparable sensitivity per the laboratory's internal validations and meet the Combined DNA Index System's (CODIS) core loci requirements[12,13]. Amplification was conducted in accordance with the respective manufacturer's guidelines.

Capillary electrophoresis was performed on an Applied Biosystems™ 3500×L Genetic Analyser. Data collection was accomplished with the 3500×L Series Data Collection software v.4 and analysed using GeneMapper® ID-X software v1.4. STRmix™ v2.6.02 software was the probabilistic genotyping software used to calculate the likelihood ratio (LR). The reported LR was derived from the Expanded Federal Bureau of Investigation (FBI) DNA population database (2016) for the Caucasian, Southeast Hispanic, Southwest Hispanic, and African American/Bahamian/Jamaican populations with a theta value of 3.0% was employed. The stratified LR, incorporating all four population groups, was selected for reporting.

The quantitation values obtained were also assessed; including: if the autosomal values met or exceeded the laboratory requirement to proceed with normalization and amplification, and the total human DNA quantity. The profile suitability for comparison, and when possible, subsequent comparison to the known owner of the firearm were evaluated. Evaluation of the data followed the standard operating procedures of an accredited forensic casework DNA laboratory. Human ethics approval was obtained for this research from Griffith University (GU ref no: 2022/275).

3. Results and discussion

3.1 Quantitation

Quantitation plays a clear role in determining if samples are suitable to move forward for amplification. In this study, it also demonstrates which areas of the firearm typically yield more DNA and demonstrates the potential DNA yield variation between donors. The final elution for the manual extraction process is approximately 30 µL and 2 µL is used for quantitation. The total amount of DNA (ng) obtained was calculated by taking the value in ng/µL obtained from the Promega PowerQuant® system and multiplying that amount by 30. Only the total autosomal DNA was assessed as the total male DNA quantities do not offer meaningful information in this particular research.

The total autosomal quantity (ng) obtained were evaluated against the three different areas sampled: the frame and slide area, the trigger and trigger guard area, and the front and rear sights area. Note, the frame and slide area from Gun C initially had a result of 0 for the autosomal target despite having values for the degradation target and the male target. This sample was re-quantified as is standard practice at the research laboratory due to the unintuitive result, the second quant value was maintained for analysis. The frame and slide area had the highest yields as expected given the larger surface area sampled. The average total yield by area was 0.80 ng for the frame slide area, 0.10 ng for the sights, and 0.13 ng for the trigger and trigger guard. When employing a cut-off of 0.001 ng/µL,

14.58% of the samples ($n = 7$) would not have been suitable for amplification based on the laboratory's casework quantitation cut-off for the DNA profiling systems.

The sum of the total autosomal DNA obtained from each firearm (all three areas combined) was also evaluated. This variation in total autosomal quantity of DNA obtained, from just 81.00 picograms for Firearm G to approximately 5.00 ng for Firearms E and O, demonstrates how biology and human behaviour can potentially play an important role in the DNA yields obtained. This large variation in DNA recovery yields has been noted in previous research, even when fewer donors were utilized[5]. Routine cleaning of the firearm, the method of cleaning of the firearm, cleaning products utilized for cleaning, and shedder status;[14–16] to name a few, can impact the quantity of biological materials that are both deposited and persist on a firearm and can subsequently be recovered. Additionally, it should be noted that these are firearms from law enforcement personnel and as such are routinely holstered or in a lockbox. One participant disclosed that they had cleaned their firearm prior to arriving for sample collection; however, due to the necessary anonymization of samples after collection, this could not be correlated with a specific firearm. Criminal firearms may be stored within the waistband of pants or undergarments, pockets, and/or loose in their environment. The more skin to skin contact with a firearm will inherently result in higher yields of DNA, while routine cleaning will reduce the persistence of deposited biological material. Sharing firearms between multiple individuals may also affect the quantity of DNA obtained[4].

In addition to the variation in the total DNA obtained from each firearm, there was also significant variation in the yields from the separate areas of the firearm (Figure 1). This is to be expected given the use of the firearm; for example, if the trigger has never been engaged it is reasonable to theorize there may be less DNA obtained from that area whereas the frame and slide area may be routinely engaged from regular firearm

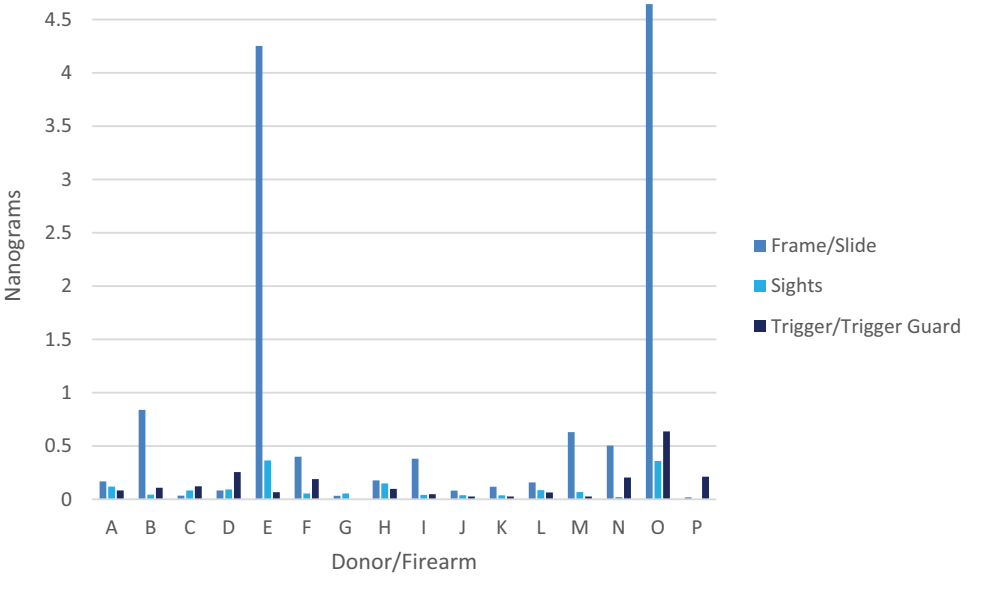

Figure 1. Sum of total DNA in nanograms recovered from each of the three areas tested on each firearm.

handling. Variation in the total yields from each area was also observed in previous research and varied based upon the use of the firearm; i.e. shooting vs just handling[4]. Total surface area will also play an important role in the yields of DNA, as the surface area of the frame and slide is significantly larger than the trigger and trigger guard or sight areas of the firearms.

3.2 Amplification and DNA typing results

All samples were amplified including samples below the 0.001 ng/μL laboratory threshold. Sample concentration was not utilized prior to amplification. It should be noted, two samples had no autosomal or male DNA detected at quantitation. The amplified samples were assessed for suitability for comparison with approximately 60.41% being suitable for comparison ($N = 29$). The determination of suitability of each DNA profile for comparison is based upon the laboratory's standard operating procedures. One requirement to be considered suitable for comparison, is a minimum number of loci, where six non-sex-determining loci must have been detected above analytical threshold. Full representation is not required at each locus, potential dropout of the sister allele is allowed.

Five samples resulted in no DNA profile obtained. This is expected, as two profiles had no DNA detected and eight total samples did not meet the laboratory cut-off to move forward for amplification. Samples not meeting laboratory cut-offs to proceed to amplification was observed in comparable research[4]. Half of the samples were deemed single source profiles ($N = 24$). Two-person mixture samples were second at approximately 39.5% ($N = 19$). No mixtures greater than two-person were obtained. Casework firearm samples will often result in mixtures of three to five persons or more, demonstrating that the population for this sample set exhibits different behaviour than that observed in some casework samples. Further research on civilian owned firearms and DNA data from firearms sampled in criminal casework may need to be explored to determine how the results of these law enforcement owned firearms may or may not differ.

The presence of a single allele(s) consistent with the firearm donor is not necessarily indicative of the incidence of that donor, but could be the result of allele sharing with an unknown donor[17]. Similarly, the absence of allele(s) consistent with a donor is not necessarily evidence of the exclusion of that individual, rather it could be allelic dropout. Therefore, profiles determined to be suitable for comparison in this study were analysed with STRmix™. The set of propositions considered were the DNA profile originated from the owner of the firearm and *N*-1 unknown individuals (where N is the estimated number of contributors in the mixed DNA profile), or the DNA profile originated from N individuals.

Hypothesis 1 (H1): Owner and N Individuals-1

Hypothesis 2 (H2): N Individuals

The LOG(LR) obtained for each owner and handler are shown in Table 1 for the 29 samples that were suitable for comparison. All sample analysed supported the DNA profile under H1, the proposition that the owner of the firearm was a contributor to the DNA profile obtained from the sample. Using the Scientific Working Group on DNA Analysis Methods

Table 1. LOG(LR) obtained for the owner of each firearm by the sampled area.

Owner	Area	Total Auto	NOC	Owner LR	Owner LOG LR	Proportion
A	Sights	0.13	2	2.52E+01	1.40	37
A	Frame/Slide	0.18	2	1.80E+05	5.26	74
B	Frame/Slide	0.90	2	1.03E+15	15.01	90
C	Trigger/Trigger Guard	0.13	1	2.21E+03	3.34	100
C	Frame/Slide	0.04	2	1.64E+15	15.22	98
D	Trigger/Trigger Guard	0.27	2	1.70E+13	13.23	97
D	Sights	0.10	2	1.01E+01	1.00	34
D	Frame/Slide	0.09	2	1.48E+08	8.17	82
E	Trigger/Trigger Guard	0.07	1	2.76E+03	3.44	100
E	Sights	0.39	1	3.85E+14	14.59	100
E	Frame/Slide	4.56	2	6.67E+16	16.82	100
F	Trigger/Trigger Guard	0.19	1	2.21E+12	12.34	100
F	Frame/Slide	0.40	1	1.14E+13	13.06	100
G	Sights	0.05	2	6.64E+03	3.82	88
H	Sights	0.15	2	5.98E+12	12.78	90
H	Frame/Slide	0.18	1	9.76E+13	13.99	100
I	Frame/Slide	0.38	1	4.86E+12	12.69	100
J	Frame/Slide	0.08	1	3.44E+05	5.54	100
K	Frame/Slide	0.12	1	3.47E+07	7.54	100
L	Trigger/Trigger Guard	0.06	1	1.78E+04	4.25	100
L	Frame/Slide	0.16	2	1.40E+12	12.15	81
M	Frame/Slide	0.63	1	6.69E+13	13.83	100
N	Trigger/Trigger Guard	0.20	2	5.19E+15	15.72	97
N	Sights	0.02	1	7.84E+04	4.89	100
N	Frame/Slide	0.50	2	5.14E+16	16.71	97
O	Trigger/Trigger Guard	0.64	2	7.63E+14	14.88	98
O	Sights	0.36	1	2.08E+09	9.32	100
O	Frame/Slide	4.65	2	4.29E+16	16.63	95
P	Trigger/Trigger Guard	0.21	2	1.84E+11	11.26	93

recommendations[18] approximately 65.5% of samples ($N = 19$) would fall into the level of 'very strong support', approximately 13.7% of samples ($N = 4$) would fall in the level of 'strong support', approximately 10.3% of samples ($N = 3$) would fall in the level of 'moderate support', and approximately 6.8% of samples ($N = 2$) would fall in the level of 'limited support'.

The estimated proportion of DNA associated with the contributor number that the LR was derived from for the person of interest is provided in the report output. The STRmix™ report provides a contributor summary with the estimated proportion of the total DNA assigned to each contributor in the profile. The LR for the person of interest in H1 will naturally be associated with the contributor that results in the highest LR. The area of the firearm, the subsequent LRs obtained for the associated samples, NOC, total autosomal DNA obtained, and proportion of mixed DNA profile as estimated by STRmix™ are shown in Table 1. The average LR obtained from the frame and slide area was 1.17E + 16, from the front and rear sights was 5.58E + 13, and from the trigger and trigger guard area was 7.46E + 14. These values are expected given the average quantities of DNA obtained from each area as well as the minimum LRs obtained from each area.

All but two samples estimated the contributor assigned with the LR associated with H1 at over 70% of the total DNA. Two samples were estimated at 34% and 37%. These two samples were collected from the front and rear sights of the respective firearm. The 34% sample (approximately 310 picogram input at amplification) had 11 loci for comparison and the 37% sample (approximately 63 picogram input at amplification) had 10 loci available for comparison. The overall quality of these two samples is reflected in the magnitude of the of the LRs obtained. The LOG of the estimated proportion of DNA of the owner's DNA in ng is plotted

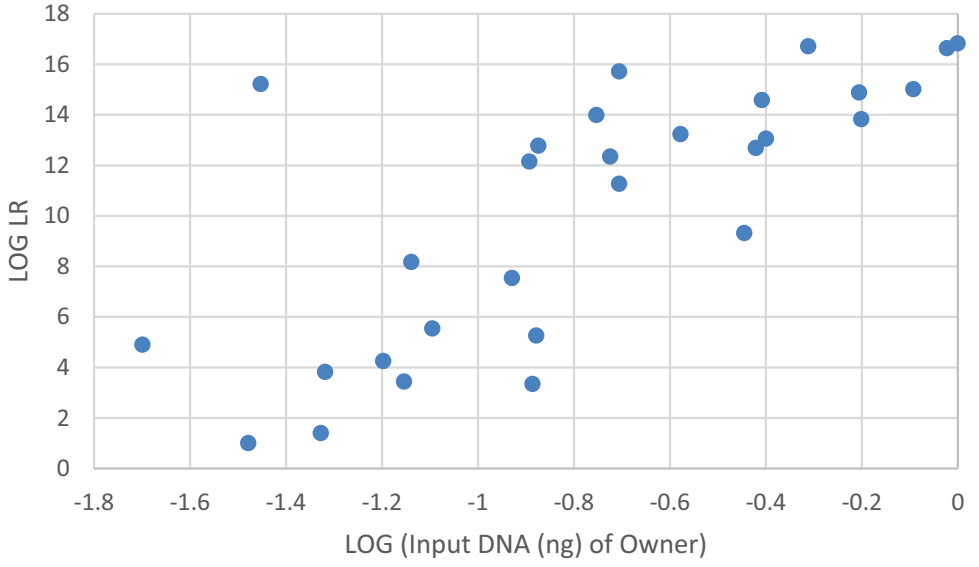

Figure 2. LOG of the estimated proportion of owner's DNA in sample (ng) versus the LOG LR for each sample.

against the respective LOG of the LR of each of the samples in Figure 2, demonstrating that as the overall contribution increases, so does the LR accordingly. The estimated quantity of input DNA of the owner was calculated by multiplying the estimated proportion of the DNA provided in the contributor summary output from STRmix™ by the total amount of DNA input in the sample for amplification and dividing by 100. It should be noted that two apparent outliers are observed in Figure 2, these are due to the relatively high proportion of the total owner DNA observed in these samples despite the low input quantity of DNA, a LOG LR of 4.89 with the proportion estimated at almost 100% and a LOG LR of 15.21 with the proportion estimated at approximately 98%, respectively. Overall, 93% of the samples suitable for comparison assigned an LR associated with a contributor estimated to be greater than 70% of the total DNA. This is consistent with previous research that found a simulated firearm owner's median relative contribution to be between 67% and 85%[4]. That same research noted a need for increased variables and increased replicates in order to be used for evaluative reporting, this research and future research aids in building upon that original dataset[4].

4. Conclusions

Caution must be taken with the interpretation of the results of this study. If a profile consistent with a person of interest is obtained from an item of evidence, they are the predominant contributor, and there is significant statistical support for the DNA profile under the proposition that includes that individual as a contributor to the DNA profile obtained it does not mean they are necessarily the owner of that firearm. That would be transposition of the conditional as well as use of the activity level fallacy[19]. However, by evaluating the evidence, and developing hypotheses, if one hypothesis is that the individual is the owner or current primary handler of the firearm, we can better analyse the types of contributions that we would

expect and use that information to evaluate the results as a whole, with competing hypotheses.

It should be stressed that this study exclusively examined law enforcement owned firearms, and that there may be variation in the results for non-law enforcement civilian owners, particularly those involved in criminal activity. Further research should be conducted to determine the differences that may or may not be expected between the different owner/handler types. However, this research did complement similar research findings regarding a simulated owner of a firearm in a controlled setting[4;] as such, additional handling scenarios and firearms recovered from criminal activity may benefit the most from further research.

Overall, if a law enforcement employee is the owner or primary handler of a firearm, and no intervention has occurred (cleaning of the firearm, deposition of a body fluid of another individual, etc.) we can expect to obtain DNA profiles that will support propositions that include the owner/primary handler as a contributor to the DNA profile obtained. Furthermore, we can also expect that more often than not, the probabilistic genotyping results will associate the LR to a contributor of a mixture that accounts for a significant proportion of the DNA obtained from that firearm, typically at least 70%. Collecting data that can aid in evaluating alternate hypotheses will assist the examiner in producing evaluative reporting results to convey their findings to the court.

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