Opportunistic crimes: Evaluation of DNA from regularly-used knives after a brief use by a different person

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Key words

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Abstract

When evaluating trace DNA recovered from evidential items in forensic casework, it is crucial to consider how the DNA got there, and such evaluative interpretations should ideally be informed by published experimental data. A key activity-level question is whether the DNA obtained comes from the regular user, the last user (ostensibly the user at the time of the crime) or from indirect transfer events. The aim of this experiment was to provide data to contribute to answering this question, particularly when considering opportunistic crimes, in which an offender might grab the nearest item at hand required for their purpose, e.g. a weapon or tool, and therefore only handle it very briefly. Volunteers ('regular users') used knives in a prescribed manner to simulate regular use (one user per knife); DNA recovery by mini-tapes from these knives gave ~1-10 ng DNA, with <16% non-donor DNA from indirect transfer events. Different volunteers ('second users') then stabbed replicate sets of regularly-used knives into a foam block for either 2, 30 or 60 sec (on different occasions), with each timeframe in triplicate, and DNA was recovered from the knife handles using mini-tapes. For knives regularly-used by three of the four volunteers, the ratios of regular user to second user DNA were approximately 4:1, 2:1 and 1:1 for durations of use by the second user of 2, 30 and 60 sec, respectively. Analysis of the respective quantities of DNA showed that this trend resulted from a

decrease in regular user DNA via transfer to the second user's hands, rather than an increase in DNA deposition from the second user. However, for knives regularly-used by the fourth volunteer, DNA from the regular user remained at significantly higher quantities than DNA from the second user and unknown sources, irrespective of duration of use by the second user. Furthermore, one volunteer deposited a similar amount of DNA through regular use as the amount of indirectly-transferred unknown DNA deposited by another volunteer's hands. These observations indicate that caution should be taken when relying solely on absolute quantities of DNA to inform evaluative interpretations, and other parameters, such as profile quality and relative contributions to mixed profiles, should also be taken into account. To better assist activity level assessments, more extensive studies of this manner should be conducted to obtain probability distributions of different types of profiles resulting from this kind of activity.

1. Introduction

When evaluating trace DNA recovered from evidential items in forensic casework, it is now widely accepted that considerations at the activity level, that is how the DNA got there, are crucial. It is recommended that such activity level evaluations be informed by empirical data with that data coming from published (peer-reviewed) structured experiments where possible [1-4]. A key activity-level question when examining items in forensic casework for so-called "touch DNA" (i.e. DNA assumed to have been deposited during an action of touching), or trace DNA, is whether the DNA obtained comes from the regular user, the last user (ostensibly the user at the time of the crime) or from indirect transfer events. The aim of the experiment presented here is to produce data to contribute to answering this question.

There are a number of published studies that have started to address the general issue of interpreting DNA findings when there are multiple users of an item [5-16]. These studies fall into three categories: the touching or handling of clean surfaces by multiple individuals [5, 7, 8, 15], the regular use of fabric items (e.g. clothing, bedding etc.) that are then used by a different individual [6, 9-13], and the regular use of items made of hard non-porous substrates (e.g. pens, keyboards, screwdrivers etc.) that are then used by a different individual [9, 12, 14, 16]. It is this latter category that we are focusing on

here, as we consider the specific issue regarding the use of hand-held items (e.g. those that are comparable to items used as weapons or burglary tools) that have been regularly used by one individual and then used by a different 'one-off' user. To our knowledge, there are only three published studies that address this specific issue [9, 12, 16], which emphasises the need for research into this topic. Research is required to investigate the factors (e.g. shedder status and manner, frequency and duration of handling by the regular or second user) that may impact the DNA results obtained to understand how these factors can be accommodated in casework evaluation, where the specific details of the incident are likely to be unknown. Research is also required to provide the data to generate probability distributions of different types of profiles resulting from this kind of activity, and, with so few studies available addressing this specific topic, more extensive studies are needed before consistent results characterised by the majority of studies can be elucidated.

Pens have been used as hard, non-porous, plastic surfaces to represent surfaces encountered as tools or weapons in casework [9, 12]. In one study, the 'regular user' vigorously rubbed plastic pens for 30-60 sec on four consecutive days to give a total of 3.5 min of use, and then second users wrote with the pens for various durations [12]. Approximately equal proportions of DNA from the regular and second users were observed when the second users wrote with the pens for 1-30 min, with a greater contribution of DNA from the second users being observed when duration of use increased to more than 30 min [12]. In another study, pens were handled by the regular user for a minimum of 20 min per day for 10 consecutive days to give an average total of 240 min of use, and then the second users handled the pens for 5, 30 or 120 min [9]. Similar findings were observed in which approximately equal proportions of regular and second user DNA were recorded at 30 min of use by the second user, with the second user DNA tending to a major profile at 120 min of use [9]. Whilst this study reported that DNA from the regular user tended to be the major profile at 5 min of use by the second user [9], which differed from the findings by van Oorschot *et al.* [12], this is presumably due to the difference in total duration of initial use by the regular user (i.e. 240 min versus 3.5 min).

However, in an opportunistic crime, an offender might grab the nearest item at hand, e.g. a weapon or tool, required for their purpose in that moment, and therefore handle it for a shorter timeframe of seconds rather than minutes. Pfeifer & Wiegand [16] start to address this by considering the effect of a second user handling burglary tools (screwdrivers, hammers and crowbars) for 30 sec after initial use by the 'owner'. They also investigated the impact of different types of handling by the second user, by considering how a burglar might intensely handle the tool to break into a property versus how the tool would normally be used. They found that DNA tended to be recovered from the second user rather than the owner when the tool was intensely used by the second user, although the owner only used the tools for 30 sec prior to use by the second user [16]. Here, we created 'regularly-used' knives in the same manner as Meakin *et al.* [17], such that the regular user handled knives for a total of 4 min across two days prior to use by a second individual, which is comparable to the study with multiple users by van Oorschot *et al.* [12]. The knives were then stabbed for 2, 30 or 60 sec by a different individual (second user) to assess the impact of shorter timeframes of second use on the DNA results obtained. In addition, we assessed the DNA data to consider whether changes in respective proportions of DNA from the regular and second users, with increased duration of use by the second user, are due to an increase in second user DNA deposition or a reduction in the persistence of regular user DNA.

2. Materials and Methods

2.1 Materials and volunteers

Plastic-handled steak knives and stabbing apparatus (consisting of a plastic box containing a foam block covered in foil) were prepared and cleaned of DNA as described by Meakin *et al.* [17]. Four participants, denoted A, B, C and D, were selected from those who volunteered on the basis of availability to attend the laboratory at the times required. Each volunteer gave informed consent to participate, and provided a buccal swab from which a reference DNA profile was generated.

2.2 Experimental set-up

Before participating in the study, the four volunteers were instructed not to have contact with each other and to avoid touching any shared items for the duration of the study. The participants were also directed not to use anti-bacterial gels and not to wash their hands in the hour immediately prior to laboratory visits.

Simulated 'regularly-used' knives were set up as described by Meakin *et al.* [17], such that each knife was handled by a single volunteer (referred to as the 'regular user') for a total of 4 min across two days. For each volunteer, three regularly-used knives were mini-taped to provide DNA samples as positive controls and three regularly-used knives were prepared each week for three consecutive weeks to give a total of 36 knives used in the following handling experiments.

Each participant attended the laboratory individually for the three consecutive days after the preparation of regularly-used knives to give ~24 h between each visit. During attendance, the participant (referred to as the 'second user') selected a knife that had been regularly used by a different volunteer and then stabbed it into the stabbing apparatus at a rate of 1 stab per 2 s [17]. During each week, participants stabbed for 2, 30 or 60 s, with a different knife used each day to give triplicate results for each duration of stabbing. DNA was recovered from each knife handle using a mini-tape within an hour of each stabbing event. The volunteers were paired at their convenience, such that when volunteers A, B, C and D were the regular users of the knives, volunteers B, A, D and C were the second users, respectively. The pairings remained the same throughout this study.

2.3 Processing of DNA samples

Extraction, quantification and profiling of DNA from the mini-tapes, and extraction and profiling of DNA from the buccal swabs, were performed as described by Meakin *et al.* [17]. In brief, the QIAamp® DNA Investigator Kit (QIAgen, Germany), the Quantifiler® Human DNA Quantification Kit (Applied Biosystems, USA), and the AmpF*l*STR® NGM SElect™ PCR Amplification Kit (Applied Biosystems, USA) using 10 µl DNA extracts were used for the mini-tape samples. DNA extracts from each individual knife handle

were quantified in duplicate to enhance accuracy; averages of these duplicate quantifications are used in Fig. 1a and 3. The buccal swabs were processed using the SwabSolution™ Kit (Promega, USA) with 2 µl extracts profiled using NGM SElect™. The 30 cycle protocol was used for all samples and PCR products were then separated using the DNA Analyzer 3730*xl* (Applied Biosystems, USA). DNA profiles were generated using GeneMapper® 4.0 software with a 100 rfu peak height threshold, as per the laboratory's internal validation study.

2.4 Data analyses

Relative contributions of DNA from the regular user, second user and any non-donor sources (referred to as 'unknown' DNA) to the profiles obtained from the knife handles were determined by comparison to the reference DNA profiles of the regular and second users. These calculations used the relative peak height contributions from the unique alleles that could be attributed to each of the respective reference profiles at each locus, and averaged across the STR loci and across the three replicates per sample [17]. To determine the amount of DNA deposited by each user, the relative contributions were multiplied by the total amount of DNA recovered for each sample. Where the minimum numbers of contributors are stated, these were determined with consideration of both number of alleles and respective peak heights. SPSS® Version 22 (IBM) was used to examine any trends in or differences between datasets. Datasets per individual volunteer were normally distributed according to the Shapiro-Wilk test ($p > 0.05$), enabling parametric statistics to be used for comparisons between volunteers and for investigating correlations with duration of stabbing. When data from different volunteers were combined (for example, to compare amounts of DNA detected from the regular user versus from unknown sources), these datasets were not normally distributed ($p < 0.05$), such that the Mann-Whitney U test was used.

3. Results

3.1 DNA recovered from knife handles after simulated regular use

Prior to the handling experiments, knives that had been handled in a manner to simulate regular use were examined for DNA. The mean quantities of total DNA retrieved from

these knives used only by volunteers A, B, C and D were 5.9, 9.1, 1.2 and 7.2 ng, respectively; the quantities recovered for each of the three replicate knife handles are shown in Fig. 1a. These total DNA quantities varied both across replicates for the same volunteer and among samples obtained from different volunteers (Fig. 1a; ANOVA $F = 4.712$, $p < 0.05$). Pairwise tests using the Student's *t*-test revealed DNA samples from knives handled by volunteers A, B and D were not significantly different, as also indicated by the range of quantities shown in Fig. 1a. However, samples recovered from knives handled by volunteer C contained significantly less DNA than those from the other volunteers ($p < 0.05$ for the three comparisons; Fig. 1a).

To calculate the quantities of DNA deposited through direct handling of the knives versus via indirect transfer events, the proportions of the DNA profiles obtained that could be attributed to the regular user versus unknown sources of DNA were first determined. These showed that, of the DNA profiles from the knives handled by volunteers A, B and D, 91-97% came from the regular user with 3-9% coming from unknown sources (Fig. 1b). The minimum number of contributors to the unknown component of these profiles ranged from 1 to 2 with a mean of 1.0. A higher proportion of unknown DNA at 16% was observed for knives handled by volunteer C (Fig. 1b), which was attributed to DNA from their romantic partner, as previously observed when volunteer C participated in a previous study (as volunteer X in [17]). When the total DNA quantities recovered were multiplied by these proportions, the mean quantities of DNA attributed to the regular user were 5.7, 8.3, 1.0 and 7.0 ng, and those attributed to indirectly-transferred DNA were 0.19, 0.86, 0.20 and 0.19 ng, for the knives handled by volunteers A, B, C and D, respectively. The range of quantities are shown as individual data points for each replicate knife handle in Fig. 1a. Comparison of the DNA quantities attributed to the regular user with those from unknown sources, across all four volunteers' samples combined, showed that significantly more DNA was deposited from the regular user than from unknown sources (Mann Whitney $U = 12.0$, $p < 0.001$). For the DNA attributed to the regular user, full profiles were observed for volunteers A, B and D, but some allele drop-out was observed for volunteer C to give partial profiles of 83-93%.

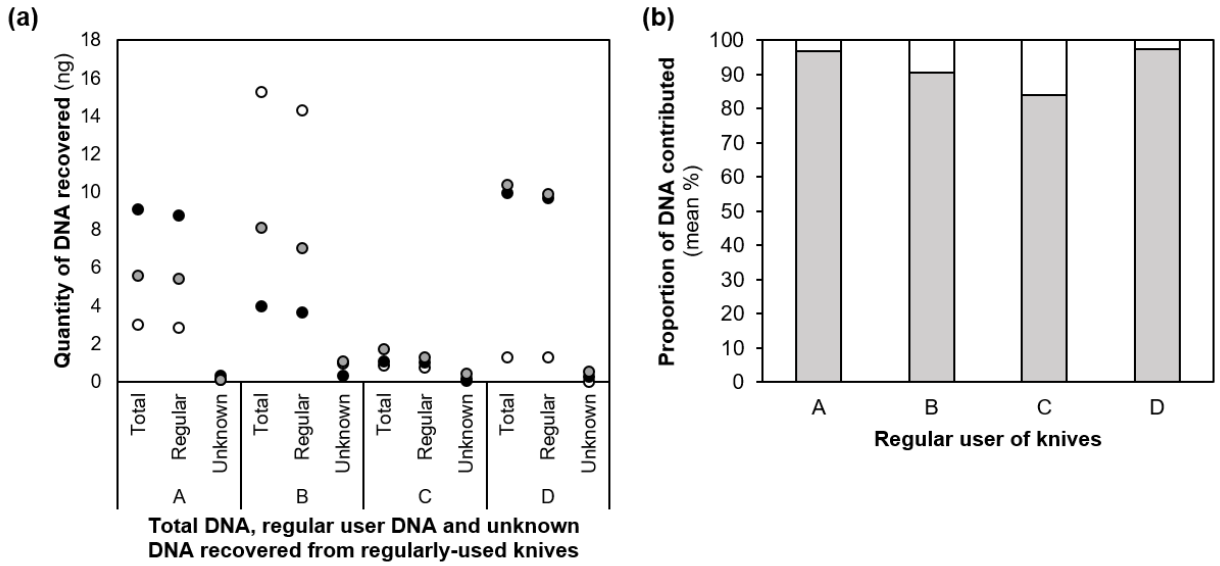

Fig. 1. Quantities of DNA (a) and respective proportions of DNA (b) contributing to the DNA mixtures recovered from the handles of simulated regularly-used knifes. In (a), quantities of DNA are from the three replicate knives (white, grey and black dots) corresponding to the total DNA recovered, the DNA attributed to the regular user, and the DNA attributed to unknown sources. In (b), proportions of DNA are means of three replicate knives contributed from the regular user (light grey bars) and other unknown sources (white bars).

3.2 Respective contributions from both users to mixed DNA profiles obtained

Each 'regularly-used' knife was then stabbed into the stabbing apparatus for a set period of time by a different user, and DNA recovered from the knife handles. For the knives that had been regularly used by volunteers A, C and D, when the second user stabbed the knife for 2 sec, the proportion of regular user to second user DNA was approximately 4:1 (Fig. 2a, c & d). This changed to approximately 2:1 when the second user stabbed for 30 sec, and to approximately 1:1 when the second user stabbed the knife for 60 sec (Fig. 2a, c & d). These observations showed a significant correlation for the decrease in proportion of regular user DNA with increasing duration of stabbing by the second user (Pearson r = -0.56, -0.49, -0.49 for knives initially handled by volunteers A, C and D, respectively; p<0.001). A corresponding significant correlation for the increase in proportion of second user DNA with increasing stabbing duration was also observed (Pearson r = -0.52, -0.38, -0.45 for knives initially handled by volunteers A, C and D, respectively; p<0.001). However, these correlations were not observed for knives

regularly handled by volunteer B and subsequently stabbed into the apparatus by volunteer A. For these knives, the proportion of regular user DNA remained high at 85-92%, irrespective of the duration of stabbing by the second user (Fig. 2b).

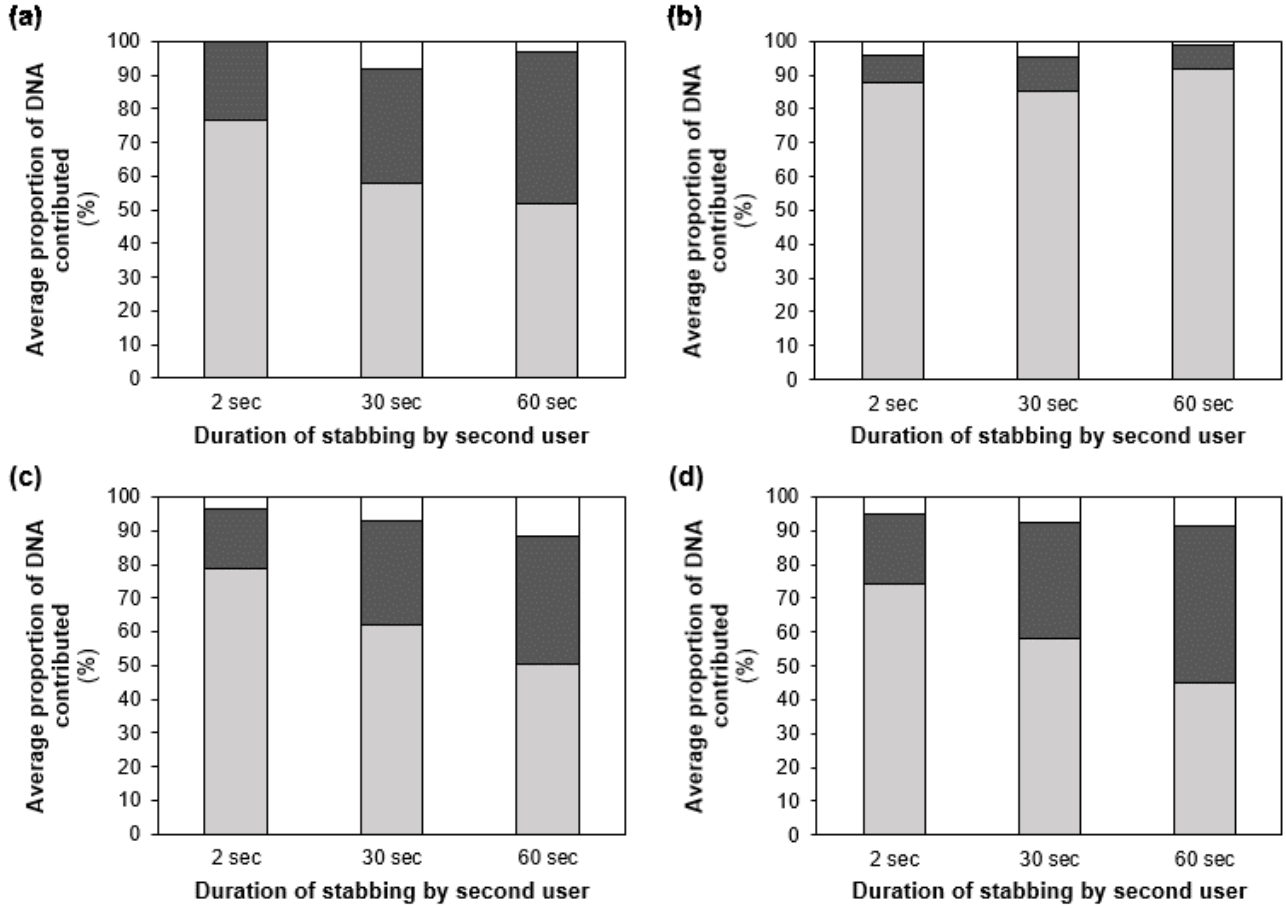

Fig. 2. Proportions of DNA contributed from the regular user (light grey bars), second user (dark grey bars) and other unknown sources (white bars) to the mixed DNA profiles recovered from knife handles that had been regularly-used by volunteers A (a), B (b), C (c) and D (d) and then stabbed in the foam block by a second user for 2, 30 or 60 sec.

3.3 DNA quantities deposited from the users and unknown sources

To determine the quantities of DNA deposited by the two users and to interrogate the observed trends further, the respective proportions of DNA in Fig. 2 were multiplied by the total quantities of DNA recovered for each knife handle. The quantities of DNA attributed to the regular user, second user, and unknown non-donor sources are presented individually for each replicate knife handle in Fig. 3. For the knives initially

handled by volunteers A, C and D, although the quantities of DNA recovered from the regular user are quite varied, they appear to decrease with increasing duration of stabbing by the second user (Fig. 3a, c & d). However, although also varied, the quantities of DNA from the second user appear to stay at similar levels, irrespective of the duration of stabbing (Fig. 3a, c & d). A Pearson's correlation was used to examine whether the quantities of DNA were significantly related to the duration of stabbing. The observed decrease in quantity of regular user DNA was significantly correlated with increasing duration of stabbing by the second user for knives initially handled by volunteers A (r = -0.55, p = 0.01), C (r = -0.61, p < 0.01) and D (r = -0.80, p < 0.001). No significant correlations were observed between duration of stabbing and quantities of DNA from the second user (p > 0.3 for all three volunteers' knives). Fig. 3b shows that no such decrease in regular user DNA was observed for volunteer B's knives; alternatively, the regular user DNA rather unexpectedly increased, particularly on the knives that were handled by the second user for 60 sec.

To consider further the amounts of DNA deposited by the two users and those coming from unknown non-donor sources, the results from the sets of knives initially handled by volunteers A, C and D were combined and examined using the Mann-Whitney U test. For these three volunteers, significantly more DNA was recovered from the regular user than the second user, when duration of stabbing by the second user lasted 2 sec (U = 42.0, p < 0.001) and 30 sec (U = 100.5, p = 0.05). However, when the stabbing lasted 60 sec, there was no significant difference between the quantities of DNA recovered from the two users (U = 132.0, p = 0.34), as per the observation of approximately 1:1 ratios in proportions of DNA from the two users (Fig. 2a, c and d). Also for these knives, the quantities of unknown DNA recovered from the knife handles were significantly lower than the DNA quantities attributed to either user (p < 0.001). For the knives regularly used by volunteer B, DNA from the regular user was recovered at significantly greater quantities than those from the second user and those from unknown sources for all durations of stabbing (p < 0.01; Fig. 2b & 3b). However, although the quantities of DNA from the second user were significantly higher than those from unknown sources for 2 and 60 sec stabbing durations (t = 2.9, p = 0.015 for 2 sec; t = 7.6, p < 0.001 for 60 sec), there was no significant difference in the quantities of DNA

recovered from the second user and unknown sources for 30 sec duration of stabbing (t = 2.1, p = 0.088). For all the knives handled by two users, the minimum number of contributors to the unknown component of the DNA profiles obtained ranged from 1 to 2 with a mean of 1.3. This is essentially the same as the minimum number of contributors to the unknown component of the DNA profiles obtained from the knives handled only by the regular user (Section 3.1). This was surprising, as a higher number of contributors might be expected in the unknown component of the second set of knives given that they were handled by two individuals, each presumably contributing DNA from separate unknown origins.

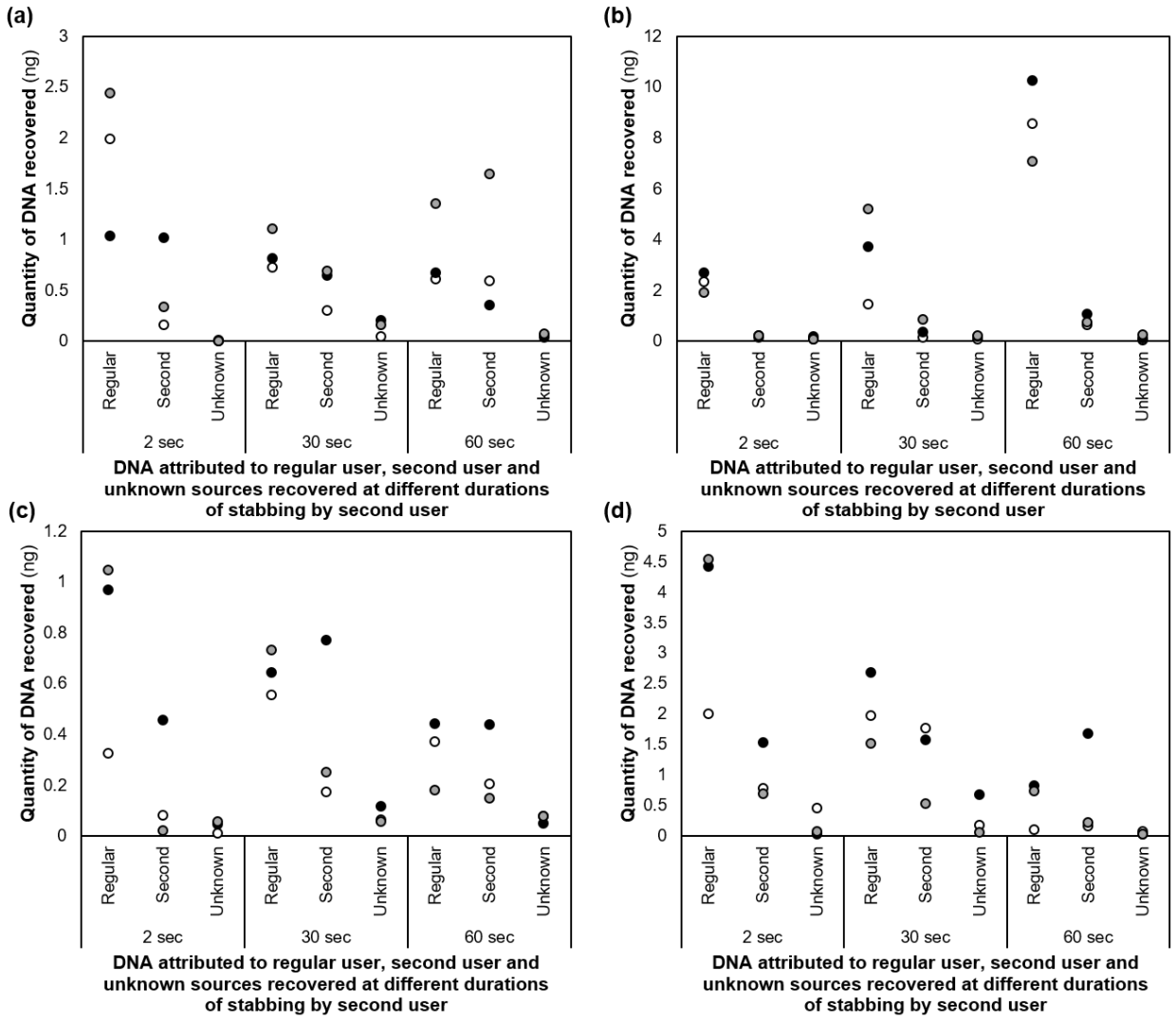

Fig. 3. Quantities of DNA attributed to the regular user, second user and other unknown sources recovered from knife handles that had been regularly-used by volunteers A (a), B (b), C (c) and D (d) and then stabbed in the foam block by a second user for 2, 30 or 60 sec. DNA quantities are presented individually from three replicate knives (white, grey and black dots).

4. Discussion

4.1 DNA deposition during regular use

During simulated regular use of the knives, volunteers deposited quantities of DNA in the 1-10 ng range, consistent with findings by previous studies for comparable non-porous items that were either regularly used in a simulated manner or actually regularly used [9, 12, 13, 17, 18]. Volunteer C deposited significantly less DNA than the other volunteers, also as previously observed when this volunteer participated as volunteer X in a prior study [17]. These observations provide further support for the concept of 'shedder status', which was first proposed by Lowe *et al* in 2002 [19] and is gaining wider acceptance in recent years [9, 17, 20-23]. DNA from unknown sources was also recovered, which had been indirectly-transferred to the knife handles via the hands of the participants. In general, this contributed to <16% of the mixed profiles obtained, as has been observed previously by numerous studies (e.g. [9, 12, 15, 17, 21]), with the slightly higher level being observed for volunteer C due to the transfer of DNA from their romantic partner.

DNA quantities are being increasingly relied upon to assist in distinguishing between different activity scenarios when evaluating trace DNA evidence [24]. Here, when the total quantities of DNA were multiplied by the relative proportions of DNA attributed to the regular user and unknown sources, the quantities of DNA deposited by the regular user were significantly greater than those deposited from indirect transfer events. This suggests that it might be possible to use such data to help distinguish between DNA deposited directly via regular use and DNA indirectly deposited via the hands of the regular users. However, it is also important to note that the amount of regular user DNA recovered from volunteer C's knives (1.0 ± 0.4 ng) is similar to the amount of unknown DNA from volunteer B's knives (0.86 ± 0.48 ng). This could be due to volunteer C being a 'poor shedder', and suggests that caution should be taken when considering whether the quantity of DNA can be used to distinguish modes of transfer, particularly when the shedder statuses of the respective DNA contributors are not known. In such situations, it might therefore be more appropriate to consider the respective proportions of the DNA observed in mixed profiles obtained, rather than the absolute DNA quantities.

4.2 Effect of increased use by second user on DNA recovered

For knives regularly-handled by three of the four volunteers (A, C and D), an apparent trend was observed showing a significant decrease in proportion of regular user DNA with a corresponding significant increase in proportion of second user DNA, as duration of stabbing by the second user increased. Whilst a similar trend has been observed by previous studies when durations of handling by the second user were minutes to hours [9, 12], these data are the first to show that this trend can also occur when handling by the second user is for just two seconds to a minute. At 30 sec of use by the second user, DNA from the regular user was still observed at a higher proportion to that from the second user, which is in contrast to the findings by Pfeifer and Wiegand [16]. This is presumably due to differences in experimental design; firstly, the manner of handling by second users varied, and secondly, second users in that study handled tools that were either genuinely regularly-used, but had not been handled for at least two weeks prior to the study, or bought new and handled once for 30 sec by the designated 'owner'. Here, regular use was simulated through the handling of clean knives for a total of 4 min across the two days immediately prior to the second user handling the knives.

Analysis of the DNA quantities attributed to the respective users revealed that the quantities of second user DNA remained similar across the knives, irrespective of the duration of use, which is consistent with the concept that increasing the duration of a single contact does not necessarily increase DNA deposition [18]. The increased proportion of DNA from the second user by 60 sec of use is therefore not due to an increase in deposition, but instead due to a decrease in the quantity of DNA from the regular user persisting on the knife handles, giving similar amounts of DNA from the two users and resulting in the approximately 1:1 ratio observed here and by other studies [9, 12]. This decrease in quantity of regular user DNA, as duration of use by the second user increases, supports a previously proposed explanation by van Oorschot *et al.* [12], that there is simultaneous transfer of DNA from the knife handle to hand, such that the hand of the second user takes increasing amounts of DNA away from the knife handles with increasing duration of use. Differences in the nature of the two substrates coming into contact, i.e. hand versus plastic knife handle, might also contribute to this finding.

Interestingly, the above change in respective proportions of DNA from the two users was not observed for the knives that were regularly handled by volunteer B and then used by volunteer A. Fig. 1a shows that the average quantity of DNA recovered from the knives that were only handled by volunteer B appeared to be higher than the other volunteers, although the t-test showed this was not statistically significant. Rather unexpectedly, Fig. 3b shows that the quantities of DNA deposited by volunteer B were higher in the samples where the knives were handled for longer durations by volunteer A. It is not clear why the quantities of DNA deposited by volunteer B varied in this manner; it may be an artefact of the intra-person variation in DNA deposits observed by Goray *et al.* [21], even though the volunteers handled the knives at similar times of day and were directed to handle the knives in the same manner each time. However, these higher quantities of DNA from volunteer B may be the reason why increasing the duration of use by volunteer A did not result in the decrease in regular user DNA observed in the other pairings of volunteers. Comparison of Fig.s 1a and 3 also shows that volunteer A deposited less DNA during the second handling experiment, than during the positive control sampling for regular use, which may also contribute to this finding. Correspondingly, the quantities of regular user DNA for volunteer B remained significantly greater than those from the second user (volunteer A) and those from unknown sources for all durations of use.

4.3 Further consideration of DNA quantities from direct and indirect transfer

For the knives that were regularly handled by volunteers A, C and D and then used by a second user, indirectly-transferred unknown DNA was observed at significantly lower quantities than DNA from both users. This supports the observations that, when examining regularly-used items, DNA that has been transferred indirectly is detected at lower levels than that which has been directly transferred [9, 12], even during an experiment purposefully designed to investigate indirectly-transferred DNA [17]. However, for the knives that were regularly handled by volunteer B, although DNA from the regular user was deposited at significantly greater amounts than indirectly-transferred unknown DNA, second user DNA was only recovered at significantly greater quantities than indirectly-transferred unknown DNA at 2 and 60 sec stabbing durations. For this pairing of volunteers, at 30 sec duration of use by the second user, similar quantities of DNA from the second user and unknown sources were recovered. This further

demonstrates that there are occasions when caution should be taken when relying solely on DNA quantity to distinguish between modes of transfer.

4.4 Concluding remarks

DNA recovery from knives that were regularly-used in a simulated manner support prior observations that regular use of non-porous items deposits ~1-10 ng DNA, with variation depending on the donor's 'shedder status', and includes <16% non-donor DNA from indirect transfer events. The use of knives, initially regularly-used by three of four participants, for just 2-60 sec by a second user resulted in a decrease in contribution from regular user DNA with a simultaneous increase in contribution from second user DNA. Analysis of the quantities of DNA contributed revealed that this trend is due to a decrease in regular user DNA via transfer to the second user's hands, rather than an increase in DNA deposition from the second user. This should be investigated further using a larger sample size of participants and 'real-life' regularly-used knives and other non-porous items. In particular, participants that vary in their shedder status should be used, given that when knives were initially regularly-used by a volunteer who could be considered a 'good shedder', subsequent use by a second user did not reduce the DNA present from the regular user, such that the proportion remained high and the quantity of DNA from the regular user remained significantly greater than that from the second user.

The data reported herein contribute to the data available for use in the determination of likelihood ratios addressing activity-level evaluations of DNA evidence in forensic casework, such as in cases of opportunistic crimes, when an offender might only briefly handle the nearest item at hand, e.g. a weapon or tool, required for their purpose in that moment. However, whilst the general trend described above was identified, it is important to acknowledge that, even with just four participants in this experiment, a deviation from that general trend was observed with knives regularly handled by one of the four volunteers. Furthermore, one volunteer deposited a similar amount of DNA through regular use as the amount of indirectly-transferred unknown DNA deposited by another volunteer's hands. These observations indicate that caution should be taken when relying solely on absolute quantities of DNA to inform evaluative interpretations. As has

been discussed previously [17], other parameters, such as profile quality and relative contributions to mixed profiles, should also be taken into account.

Complex and variable scenarios are frequently encountered in forensic science and being able to offer reproducible and transparent inferences is important for robust forensic reconstructions [2]. The data presented in this study illustrate the broad value of developing casework informed empirical studies that can provide data to underpin evaluative interpretations of activity level propositions [4, 25]. To better assist activity level assessments, more extensive studies of this manner should be conducted to obtain probability distributions of different types of profiles resulting from this kind of activity. This will also enable an elucidation of consistent results characterised by the majority of studies for use in casework.

Conflicts of interest

None.

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References

[1] Association of Forensic Science Providers. Standards for the formulation of evaluative forensic science expert opinion, Sci Justice. 49 (2009) 161-164.

[2] G. Tully. Forensic Science Regulator Annual Report (2017) Available online at: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/674761/FSRAnnual_Report_2017_v1_01.pdf.

[3] European Network of Forensic Science Institutes. ENSFI Guideline for Evaluative Reporting in Forensic Science: Strengthening the Evaluation of Forensic Results across Europe. (2015) Available online at: http://enfsi.eu/wp-content/uploads/2016/09/m1_guideline.pdf.

[4] R.A.H. van Oorschot, B. Szkuta, G.E. Meakin, B. Kokshoorn, M. Goray. DNA transfer in forensic science: A review, Forensic Science International: Genetics. 38 (2019) 140-166.

[5] M.K. Balogh, J. Burger, K. Bender, P.M. Schneider, K.W. Alt. STR genotyping and mtDNA sequencing of latent fingerprint on paper, Forensic Sci Int. 137 (2003) 188-195.

[6] M. Breathnach, L. Williams, L. McKenna, E. Moore. Probability of detection of DNA deposited by habitual wearer and/or the second individual who touched the garment, Forensic Sci Int Genet. 20 (2016) 53-60.

[7] A.K. Buckingham, M.L. Harvey, R.A.H. van Oorschot. The origin of unknown source DNA from touched objects, Forensic Sci Int Genet. 25 (2016) 26-33.
[8] M. Goray, R.A.H. van Oorschot. The complexities of DNA transfer during a social setting, Leg Med. 17 (2015) 82-91.
[9] F. Oldoni, V. Castella, D. Hall. Shedding light on the relative DNA contribution of two persons handling the same object, Forensic Sci Int Genet. 24 (2016) 148-157.
[10] S.F. Petricevic, J.A. Bright, S.L. Cockerton. DNA profiling of trace DNA recovered from bedding, Forensic Sci Int. 159 (2006) 21-26.
[11] J.J. Raymond, R.A.H. van Oorschot, S.J. Walsh, C. Roux, P.R. Gunn. Trace DNA and street robbery: A criminalistic approach to DNA evidence, Forensic Sci Int Genet Suppl Ser. 2 (2009) 544-546.
[12] R.A.H. van Oorschot, G. Glavich, R.J. Mitchell. Persistence of DNA deposited by the original user on objects after subsequent use by a second person, Forensic Sci Int Genet. 8 (2014) 219-225.
[13] M. van den Berge, G. Ozcanhan, S. Zijlstra, A. Lindenbergh, T. Sijen. Prevalence of human cell material: DNA and RNA profiling of public and private objects and after activity scenarios, Forensic Sci Int Genet. 21 (2016) 81-89.
[14] A.E. Fonneløp, H. Johannessen, P. Gill. Persistence and secondary transfer of DNA from previous users of equipment, Forensic Sci Int Genet Suppl Ser. 5 (2015) e191-e192.
[15] B. Szkuta, K.N. Ballantyne, B. Kokshoorn, R.A.H. van Oorschot. Transfer and persistence of non-self DNA on hands over time: Using empirical data to evaluate DNA evidence given activity level propositions, Forensic Sci Int Genet. 33 (2018) 84-97.
[16] C.M. Pfeifer, P. Wiegand. Persistence of touch DNA on burglary-related tools, Int J Legal Med. 131 (2017) 941-953.
[17] G.E. Meakin, E.V. Butcher, R.A.H. van Oorschot, R.M. Morgan. Trace DNA evidence dynamics: An investigation into the deposition and persistence of directly- and indirectly-transferred DNA on regularly-used knives, Forensic Sci Int Genet. 29 (2017) 38-47.
[18] R.A.H. van Oorschot, M.K. Jones. DNA fingerprints from fingerprints, Nature. 387 (1997) 767.
[19] A. Lowe, C. Murray, J. Whitaker, G. Tully, P. Gill. The propensity of individuals to deposit DNA and secondary transfer of low level DNA from individuals to inert surfaces, Forensic Sci Int. 129 (2002) 25-34.
[20] A.E. Fonneløp, M. Ramse, T. Egeland, P. Gill. The implications of shedder status and background DNA on direct and secondary transfer in an attack scenario, Forensic Sci Int Genet. 29 (2017) 48-60.
[21] M. Goray, S. Fowler, B. Szkuta, R.A.H. van Oorschot. Shedder status - An analysis of self and non-self DNA in multiple handprints deposited by the same individuals over time, Forensic Sci Int Genet. 23 (2016) 190-196.
[22] S.H.A. Tobias, G.S. Jacques, R.M. Morgan, G.E. Meakin. The effect of pressure on DNA deposition by touch, Forensic Sci Int Genet Suppl Ser. 6 (2017) e12-e14.
[23] P. Kanokwongnuwut, B. Martin, K.P. Kirkbride, A. Linacre. Shedding light on shedders, Forensic Sci Int Genet. 36 (2018) 20-25.
[24] D. Taylor, A. Biedermann, L. Samie, K.M. Pun, T. Hicks, C. Champod. Helping to distinguish primary from secondary transfer events for trace DNA, Forensic Sci Int Genet. 28 (2017) 155-177.
[25] R.M. Morgan. Conceptualising forensic science and forensic reconstruction. Part I: A conceptual model, Sci Justice. 57 (2017) 455-459.