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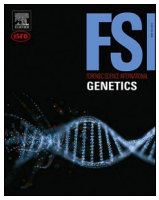

Comparison of DNA profiles from samples collected from underneath fingernails and hand deposits following everyday activity

Mariya Goray [a,*], Adrian Linacre [a], Roland A.H. van Oorschot [b,c], Duncan Taylor [a,d], Kahli Murton [a]

[a] College of Science and Engineering, Flinders University, Adelaide, SA 5042, Australia
[b] Office of Chief Forensic Scientist, Victoria Police Forensic Services Department, Macleod, VIC 3085, Australia
[c] School of Agriculture, Biomedicine and Environment, La Trobe University, Bundoora, VIC 3086, Australia
[d] Forensic Science SA, Adelaide, SA 5001, Australia

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ABSTRACT

In instances of direct physical contact between individuals involved in criminal activity, body samples can provide significant and relevant information to aid in criminal investigations and court proceedings. Fingernails are one such forensically relevant body area that is capable of providing evidence of direct contact and potentially revealing whether the interaction involved was of a forceful kind. Several studies have investigated the prevalence of non-self DNA under fingernails after different crime-related scenarios; however, few have assessed the types of DNA profiles found after everyday activities. Furthermore, the comparability of the fingernail samples to those deposited on contacted surfaces remains unknown. In this study, we examined the composition of self- and non-self-DNA in samples collected from under the fingernails and held tubes from the same set of individuals. Additionally, the potential use of fingernails samples for shedder assessment was evaluated through comparison with two common shedder categorisation tests. For these purposes, samples were collected from both hands of 25 individuals of different demographics, without any prior restrictions on activities. Direct deposits were made by holding a 50 mL tube (for DNA shedder testing) and placing index fingers onto a slide (for Diamond™ dye cell counting shedder testing). Fingernail samples from both hands were taken immediately after tube-holding deposits. Reference DNA samples were collected from the participants as well as their cohabitating partners and other adults. Qualitative and quantitative data on DNA and cell deposits were collected to support activity-level evaluations. In our study, mixture inversions were rare, with non-self DNA, when detected, usually present as a minor component. More non-self DNA was detected after participants' contact with the tube compared to fingernail samples. Partners' DNA was frequently detected in both sample types, but more so in fingernail samples. Comparisons of the three shedder testing methods (fingernails, tube holding and cell count) showed that the categorisation results of these methods are not interchangeable and that DNA methods (tube vs. fingernails) were more consistent (64 % of deposited classified into the same shedder category) with each other than with cell counts (tube vs. cell count: 52 % classified into the same shedder category) (fingernails vs. cell count: 40 % remained in the same category). We anticipate that these datasets will serve as a valuable resource for activity-level evaluations and encourage other investigators to contribute to the growing data collection.

1. Introduction

In instances of direct physical contact between individuals involved in criminal activity, body samples can provide significant and relevant information to aid criminal investigations and court proceedings. Fingernails are one such forensically relevant body area that is capable of providing evidence of direct contact and potentially revealing whether the interaction involved was forceful. DNA collected from fingernails has the potential to persist for long periods, as it is protected from environmental factors and physical removal due to its location beneath the nail bed [1]. Various conditions for the possible presence of non-self DNA under fingernails have been investigated through studies that assessed fingernail samples taken from the general population [2–6], cohabitating individuals [3,4,7,8], persons sampled after sexual contact

* Corresponding author.
E-mail address: mariya.goray@flinders.edu.au (M. Goray).

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or scratching activities [3,4,7,9,10] and casework samples [1,4,11–15]. While non-self DNA detection varied between the studies, non-self DNA was detected in up to 41 % of the samples (with 'high quality/level' mixtures, as defined by the authors, reported in up to 17 % of those) [2–6,8]. The exception was a study on digital penetration involving vaginal cells, a biological material with a relatively high quantity of DNA compared to other samples types, which resulted in 100 % non-self DNA detection immediately after the tested action of digital penetration [3]. Comparatively, the prevalence of non-self DNA on hands, or surfaces touched by hands, was higher than that under fingernails, with over 79 % of the samples found to have non-self DNA contribution [16–19]. The association of this unknown DNA with cohabitating individuals varied between the studies, where, for example, Dowlman et al., [5] identified cohabitating partners in approximately 23 % of the samples (and 55 % of the mixtures), while Matte et al., [4] did not detect them in any of the samples. However, the comparability of the frequency of detection and composition of non-self DNA on hands versus those found under the fingernails has yet to be investigated.

Furthermore, the tendency of individuals to leave traces of themselves wherever they go can have significant implications for evidence collection and interpretations of results [16]. This is particularly true for touch DNA deposition, where shedder status appears to be a key factor in both the chance of transfer and its post-transfer detectability [18,20–29]. Shedder status is a measure of an individuals' propensity to deposit DNA during physical contacts with a surface. A recent proposal [30] suggested replacing the commonly used term "shedder status" with "individual's shedding propensity". While terminological discussions can be valuable, this particular suggested change fails to offer substantive improvement to the field. The well established "shedder status" classification already accurately reflects the empirically observed differences in DNA shedding among individuals.

The shedder tests currently employed by researchers can be broadly divided into two distinct approaches: those that employ DNA profiling techniques [18,20–22,26,27] and those that use cell counts [20,23,27,28,31]. However, even within the same type of approach, significant methodological variability exists between tests, including differences in deposition surface type (plastic, fabric, glass, etc.), duration of contact, activities performed prior to deposition (such as handwashing), the DNA processing methods, the size of the deposit (whole hand vs. finger for the cell counting methods), and the means of analysing the generated data to classify an individual [20]. The impact of these variables on subsequent classification is largely unknown, but recent data suggest that differences in selected methodology can result in changes in shedder designation [20,27,31]. While shedder research has significantly advanced our understanding of touch DNA composition and origin on hands [32], the source and makeup of touch DNA beneath fingernails is less understood, especially after everyday activities. Moreover, the correlation between shedder categorisation from hand samples (or surfaces touched by hands) and those retrieved from beneath fingernails remains unexplored.

The present study explores the nature of self and non-self DNA contributions found under fingernails and handheld surfaces. It aims to determine the origin of non-self DNA (i.e. a cohabitating partner, other cohabitating adult, or another source) and whether the non-self DNA traces share a common donor. To this end, we examined the DNA profiles obtained from under fingernails and handheld plastic tubes (commonly employed in shedder studies) of 25 individuals engaged in routine, everyday activities to ascertain the possible relationship between the two. Further, the shedder designation concordance between the DNA (both fingernails and tube holding) and cell count methods was assessed, expanding on other recent studies that compared different shedder testing methods [20,27], taking into account demographic (age, sex, occupation) and activity- related (e.g. time since handwashing, use of moisturisers) factors relevant to the participants.

2. Materials and methods

2.1. Experimental design and sample deposition

Twenty-five participants, representing a broad demographic spectrum and over the age of 18, were recruited for this study comprising 12 males and 13 females. Participant demographic and pre-deposit activity information was collected via a questionnaire (Supplementary Data 2). The participants' hand size was measured from ink handprints on paper (laid over a glass plate) created by participants after ink was applied to their hand. These prints were used to measure the length and width of the hands and to classify participants into small, medium and large hand sizes (Supplementary Data 2).

Cell deposits, for cell count shedder status assessment, were performed immediately prior to DNA collection from under fingernails and tube holding. For the cell count shedder status assessment part of the study, an additional 17 sets (two fingers) of prints were collected from the participants' family members, resulting in a total of 42 sets (of two fingers) of prints (n = 84; 25 sets from participants and 17 from family members). Participants were instructed to place their left and right index fingers onto individual sterile microscope slides for 15 s. Each slide was overlaid with a 40-square or grid (1 mm×1 mm) numbered grid on its underside, to facilitate accurate cell counts (Fig. 1). No conditions or specific instructions were provided to the participants about their activities prior to sample collection.

The slides were sprayed with a 20 % Diamond™ Nucleic Acid Dye (DD) solution (Promega, Madison, USA), that was diluted with 70 % ethanol from stock solution, using Voilamart HS08 mini air compressor (Voilamart™, Sydney, AU). The stained deposits were allowed to dry for 30 min prior to image collection using DinoLight (EDGE AM4115T-GFBW, AnMo Electronics Corporation, Clarksburg, AU) microscope under 50x magnification. DinoLite EDGE AM4115T-GFBW digital microscope is equipped with a blue LED excitation source centred at approximately 480 nm and an emission filter cantered at 510 nm, facilitating the detection of green fluorescence signals consistent with Diamond Dye. Signal which may correlate to nuclear DNA was counted in order to estimate cell counts using ImageJ, a Java-based image processing program (v 1.54 f) (LOCI) [33]. For each fluorescent signal observed, cellular morphology, specifically size and shape, was assed to confirm consistency with the expected cellular characteristics at this magnification [34,35] with assistance of ImageJ. The images were converted to 8-bit grayscale to ensure colour consistency and circularity was set to 0.6–1, to allow for differentiation between cells, fibres and other artefacts. All grid squares containing cells were used to determine the total number of cells in the deposit and the average cell number (calculated as total number of cells divided by the number of grids with observed cells). Additionally, the 3 and 5 squares of the deposit grid (Fig. 1 right) with the highest cell density were imaged at 220x magnification to compare different cell counting shedder testing methods. For the latter two methods, cells in the selected 3 and 5 grid squares were counted and averaged based on the number of grids used in the count. More specifically, the 3 or 5 grids in a single print that had the most cells were imaged. All the cells in these grids were counted generating the total cell count. This total cell count was then averaged based on the number of grids used (e.g., for example for the 3-grid method, all cells in the 3 grids were counted (total cell count) and divided by 3 to get an average number of cells per grid). This allowed comparison of average cell numbers when using all grid squares with cell deposits against a portion of the deposit (either 3 or 5 grid squares) with the highest cell densities to investigate if such sub-sampling of the entire finger deposit can be used instead of the whole print.

DNA collection was performed immediately after cell deposits (for the cell count shedder assessments) by instructing the participants to hold a sterile 50 mL plastic tube in each hand for 15 s (n = 50). The DNA was collected from each tube separately via a wet and dry double swabbing technique using two Sarstedt XL forensic swabs (Nümbrecht,

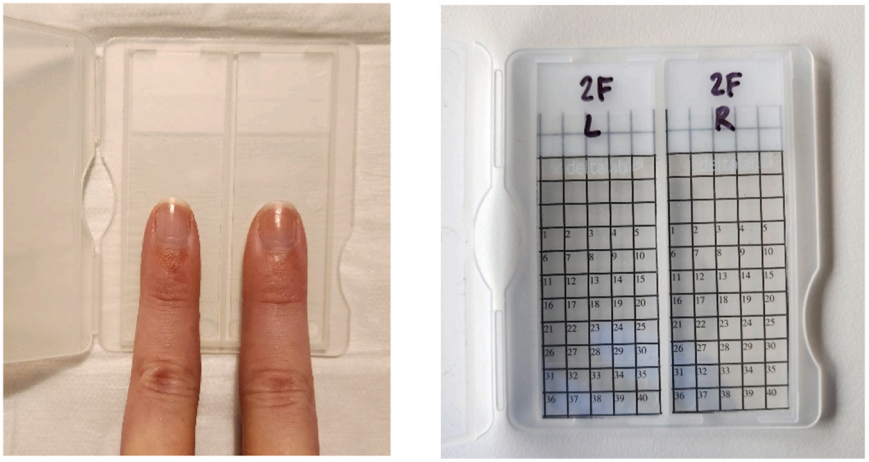

Fig. 1. Cell deposit shedder method (left) and the gridded microscope slides used during experiments (right).

Germany). The two swabs were combined for DNA analysis. Fingernail samples were taken immediately following the tube holding part of the experiment using a wet swabbing technique with a MWE Dryswab (Medical Wire and Equipment, Durham, USA) swab. All fingers of each hand were swabbed as a single sample (i.e. creating one sample from the right-hand fingernails and one sample from the left-hand fingernails) for DNA analysis ($n = 50$). Buccal DNA samples were collected from all participants and all cohabitants of the participants over the age of 18, which were identified as either being the participant's partner, the participant's child, the participant's parents and siblings or another adult (Supplementary Data 2).

All samples were collected following informed consent and with approval from relevant human ethics committee (Flinders University Ethics Committee 4915).

2.2. Sample processing

DNA was extracted with DNA IQ™ System (Promega) following the manufacturer's protocol (final elution volume was 60 μL). DNA quantification was performed using Quantifiler™ Trio DNA quantification kit (ThermoFisher Scientific, Waltham, USA) following the manufacturer's recommended protocol. STR amplification was performed using PowerPlex®21 kit (Promega) in a final volume of 25 μL. The DNA amount used in amplification was 0.5 ng, or 15 μL if the concentration was ≤ 0.033 ng/μL (30 cycles). The fragments were separated using 3500xl Genetic Analyser (ThermoFisher Scientific) using a 24 s injection at 1.2 kV. An allele detection threshold was set at 175 relative fluorescence units (RFU). The data were analysed using Genemapper ID-X (v1.6) (ThermoFisher Scientific).

2.3. Data analysis

The number of contributors to DNA profiles was determined based on the maximum allele count and peak height information. Mixture deconvolution, mixture proportion assignment and likelihood ratio calculations (no minimum threshold was used for inclusions) were performed using STRmix™ (v2.9) (New Zealand Institute of Public Health and Forensic Science and Forensic Science South Australia). The major contributor was defined as a person contributing ≥70 % of total DNA in the sample (based on STRmix™ mixture proportions). The majority contributor was defined as the individual contributing the majority of DNA (based on STRmix™ mixture proportions) where no single contributor reached 70 % contribution.

DNA quantities were log10 transformed for analysis. Possible presence of common unknown contributors was assessed via STRmix™ mixture-to-mixture comparisons (LR calculations without θ). When the donors of the samples (the participants) were not excluded as a contributor(s) (based on inclusionary LR; no thresholds were used for inclusionary LRs), these samples were deconvoluted again while conditioning on these donors. The majority of the inclusionary LRs were > 100 billion, however there were several LRs $> 100{,}000$ and 4 LRs that were less than 10. The profiles with lower inclusionary LRs (the four instances where LRs were below 10) corresponded to samples where the sample donor was a minor contributor, represented by only a few alleles.

Data normality was tested using Shapiro-Wilk test and differences between datasets were tested using Mann-Whiteny U or Kruskal-Wallis H nonparametric tests, along with bootstrapping or Dunn's post-hoc testing as required ($p < 0.05$). ANOVA and paired t-tests were performed for parametric testing ($p < 0.05$) and correlation between datasets was tested using Spearman's Rank or Pearson's Correlation. Differences in shedder categorisation between different methods was analysed using confusion matrices and Fisher's Exact test. Data analysis was performed using IBM SPSS statistical software (v 29.0.2.0; New York, USA) and R (v 4.4.1).

3. Results and Discussion

3.1. Fingernail samples

The total DNA amounts recovered from the samples taken from beneath fingernails ($n = 50$) ranged from 5.23 ng to 478.5 ng (av. 45.24 ng) and full DNA profiles were able to be generated from all samples. On average, 96 % (av. 44.72 ng, based on STRmix™ proportions) of the DNA recovered from the samples was attributed to self-DNA and 4 % (av. 0.52 ng) to non-self DNA (Fig. 2). Further, 28 % of participants did not have any non-self DNA detected for either hand. Of the 50 samples (left and right hands), almost half (46 %) were single source profiles while 52 % and 2 %, respectively, were two- and three-person mixtures. In samples where non-self DNA was detected (54 % of samples that detected 2 and 3 contributors), the majority of this non-self DNA was attributed to the participant's partner (59 %), followed by parents and children (11 %), and friends (2 %). Unknown donors were detected in 30 % of the mixtures. The mixture proportion of self-DNA ranged from 33 % to 100 % (av. 96 %), and non-self DNA from 0 % to 67 % (av. of 4 %) (Supplementary Data 1). Mixture inversion (where non-self donor was detected as the major/majority contributor) was detected in a single sample (2 % of samples) and the majority contributor was identified as the partner of one of the participants (contributing 67 % of DNA with the remaining DNA attributed to the sample donor). For the remaining samples, the participant was detected as the single source/major/majority contributor. When detected, the partners' and other family members' Likelihood Ratios (LRs) ranged from

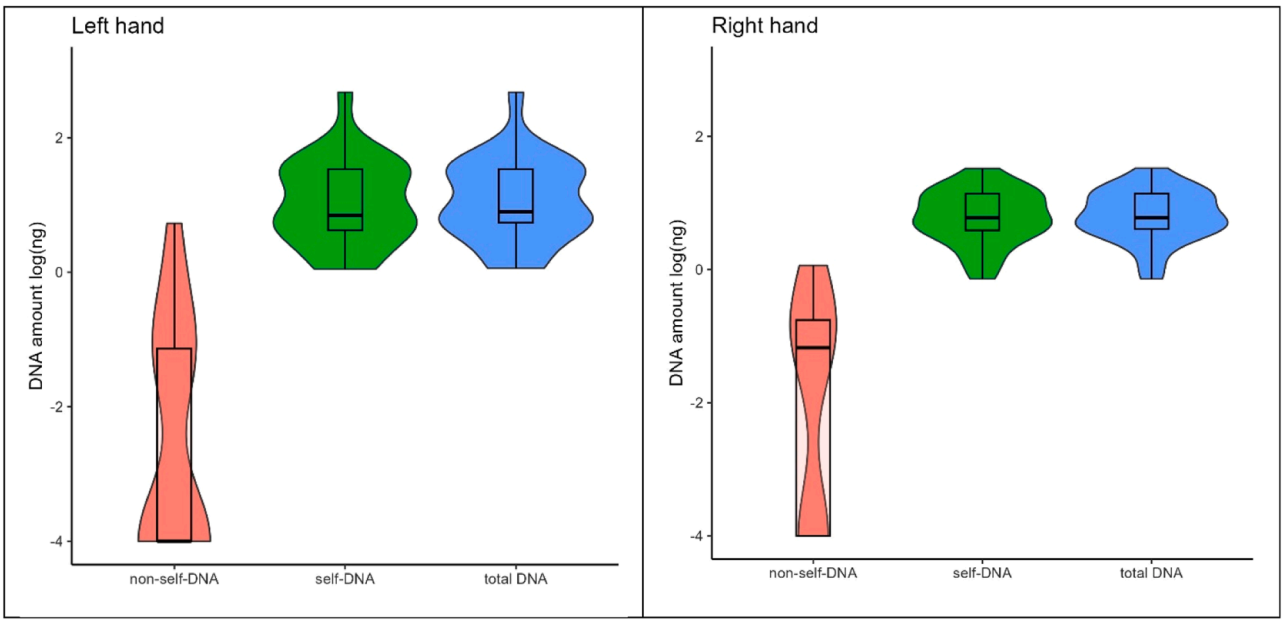

Fig. 2. Violin plot of log 10 self-, non-self and total DNA beneath fingernails of the left and right hands of 25 participants. DNA amounts of 0 have been plotted at −4.

2×10^{0}–1.97×10^{28} (median 8.29×10^{02}; av. 8.95×10^{26}). Where a partner's DNA was identified under a fingernail, it was also found on the corresponding handheld tubes in 24 % of the cases. DNA from the partner was detected under the fingernails but not on tubes in 24 % of the samples, on the tubes but not under the fingernails in 16 % and neither in 45 % of the samples.

These findings align with existing research on non-self DNA prevalence under fingernails in the general population and in the context of cohabitation [2,4–6]. However, these previous studies are over a decade old, and the use of more sensitive technologies provides fresh insights into DNA transfer and persistence in current real-world scenarios. In contrast to previous research [18,28,32,36], males did not have significantly more self DNA than females ($p = 0.068$). However, females exhibited significantly higher levels of non-self DNA compared to males ($p = 0.018$). Age has also been previously associated with differences in shedder status [37–39], with children shedding more DNA than adults. No significant differences were noted in different age groups tested when assessing the quantities of self-DNA under the fingernails (the four age groups tested included 18–35 years ($n = 6$), 36–55 years ($n = 7$), 56–75 years ($n = 7$) and 76+ years ($n = 5$)). This discrepancy may be attributed to the absence of participants under 18 years of age in our study cohort.

The impact of nail length on the DNA results was assessed by dividing all participants into four groups based on their nail length: very short (does not exceed fingertip), short (same length as fingertip), medium (exceeds length of fingertip) and long (greatly exceeds length of fingertip) (Supplementary Data 1). No significant differences were observed with either self- or non-self DNA quantities across nail lengths. Conversely, self-reported nail biting within five hours of sampling significantly increased the amount of self-DNA under the fingernails ($p = 0.014$). While saliva can persist under fingernails for up to 24 h [10], in this study, most participants washed their hands after they reported nail biting and before sampling. Similar increases in self-DNA from self-reported nail biters were not evident in their direct tube deposits. This is likely due to the area of the hand exposed to nail biting not contacting the tube during deposition and/or only forming a very small portion of the hand's deposit. Hand dominance was also found to be significant, with more self-DNA found under the non-dominant hand's fingernails ($p = 0.049$). The reasons for these findings are unknown, but it's possible that more self- contacts are made with the non-dominant hand (picking up more self-DNA) [40] or that the non-dominant hand is less exposed to actions that remove DNA [41].

The participants in this study were not given any instructions regarding pre-deposit activities, to ascertain what DNA or cellular material could be found on individuals' hands in everyday circumstances. This also included no instructions regarding handwashing or hand sanitising (Supplementary Data 2). Participants were asked when they last washed their hands and whether soap had been used. All but one (8 h) time frame fell within either the 0.5- hr category or the 2.5- hr category. Neither the time since handwashing nor use of moisturisers and sanitisers significantly altered the amounts of self- and non-self DNA, regardless of whether soap was used or not. However, the extended time frames of at least one-hour post-handwash may have masked the significance of cleaning. Furthermore, time since self-reported sexual physical activities (<30 min, 30–60 min, 60–120 min and >120 min) did not significantly affect the amount of non-self DNA detected.

No significant differences were observed between the quantities of self-DNA based on the work location (indoors vs outdoors (Mann Whitney *U* test $p = 0.26$)); however, significantly more non-self DNA was detected under the fingernails of the indoor workers compared to those who work outside (mostly farmers) (Mann-Whitney *U* test $p = 0.046$) Indoor, office workers are more likely to interact with many different individuals working in the same building and come into contact with communal spaces and items, collecting non-self DNA on their hands and under the fingernails. Conversely, farmers often work alone for extended periods of time and encounter fewer communal spaces; further research is needed to fully explore this trend.

3.2. Direct hand deposits on tubes

The total DNA amounts deposited on the tubes, separate for each hand, ranged from 0 to 18.84 ng (av. 1.51 ng) (Fig. 3) (Supplementary Data 1). Of these 50 samples, 2 % did not generate a profile, 26 % were single source profiles and 60 % and 12 % were two and three person mixtures, respectively (av. 2 contributors across all samples inclusive of left and right hand). In the majority of samples (88 %), the donor was detected as the single source/major/majority contributor. However, non-self DNA donor was detected as major/majority contributor in 12 % of the samples ($n = 6$). These six samples were attributed to four

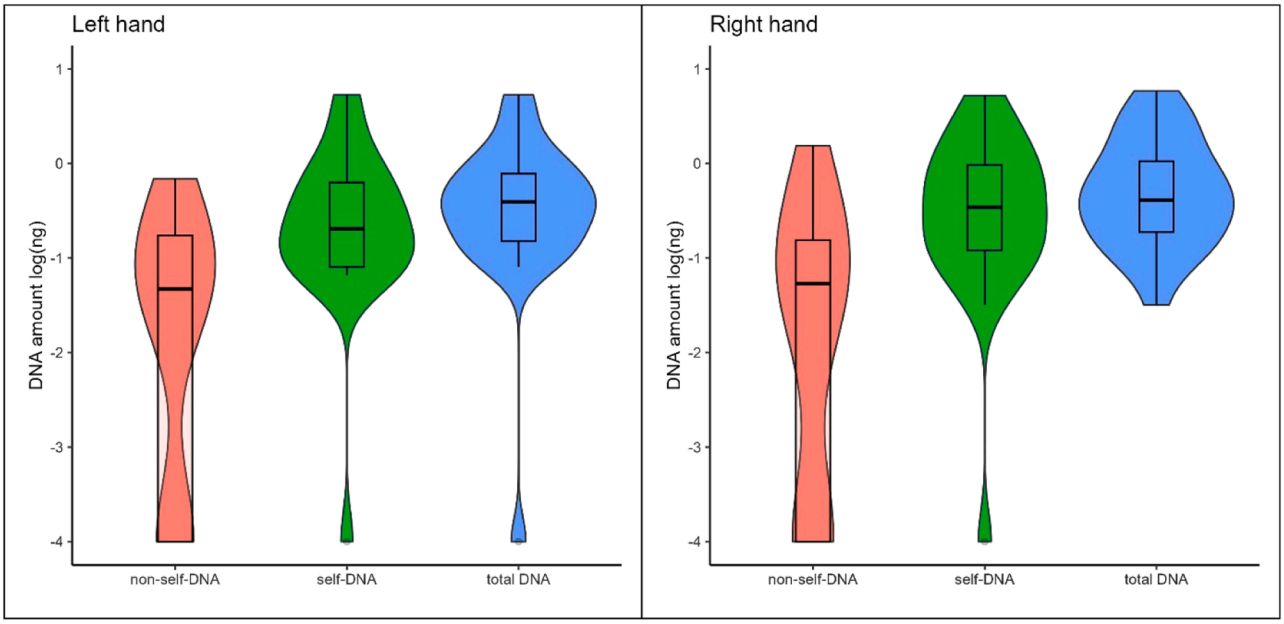

Fig. 3. Violin plot showing the distribution log 10 (ng) self-, non-self and total DNA data for the left and right hands of 25 participants, illustrating the density of the DNA quantities. Any results of no DNA have been plotted at −4.

participants (including both hands of two participants) with participants' partners not excluded from four of these samples (Supplementary Data 1). Of the 36 samples containing non-self DNA (72 % of the 50 samples), the individuals residing with the tested participants were observed in 15 (42 % of samples with non-self DNA) of the samples; with LRs ranging from 2×10^{0}–3.31×10^{25} (median 4.97×10^{04}; av. 3.56×10^{25}) (Supplementary Data 1). In general, across all samples, an average of 1.37 ng was attributed to self-DNA (based on STRmix mixture proportions) and 0.14 ng to non-self DNA.

The results of the DNA deposits on the tubes were further analysed in light of the questionnaire responses to identify possible demographic influences (Supplementary Data 1). In agreement with multiple shedder studies [18,28,32,36], males deposited significantly more self-DNA than females ($p < 0.001$); however, the differences in non-self DNA amounts on hands were not significant ($p = 0.549$). The causes of the sex-specific differences are not fully known, but various factors have been proposed to contribute, including sweating propensity [38], hygiene habits [42] and hand size. With regard to different age groups, while there appears to be a trend of reduced DNA deposition by the older individuals (Fig. 4), these differences were not significant.

Participants self-evaluated their sweating propensity, indicating if they generally had sweaty hands. Several studies suggested sweating as a contributing factor to shedding ability [29,32], and while hand sweatiness was only subjectively self-determined (rather than by some quantitative measure), it was found to be a significant factor in this study ($p = 0.041$). Additionally, in our participant group, three individuals reported existing skin conditions. Two of these participants, who had eczema and dermatitis, were found to be high shedders (Section 3.3). The third participant, who reported a facial skin condition, was classified as an intermediate shedder. Notably, the two high shedders had higher than average amounts of self-DNA under their fingernails (49.01 ng and 78.8 ng). Further, the partner of one of these two individuals (Supplementary Data 1; participants 2E and 2 F) had high levels of non-self DNA (87 %) both on the tube and under fingernails, attributed to the participant with the skin condition. These participants were not excluded from our analysis, as skin conditions are common in a population and thus represent the variety of data that one can expect when testing a diverse group of individuals. Questionnaire data indicated no recent direct physical contact between these two people. This

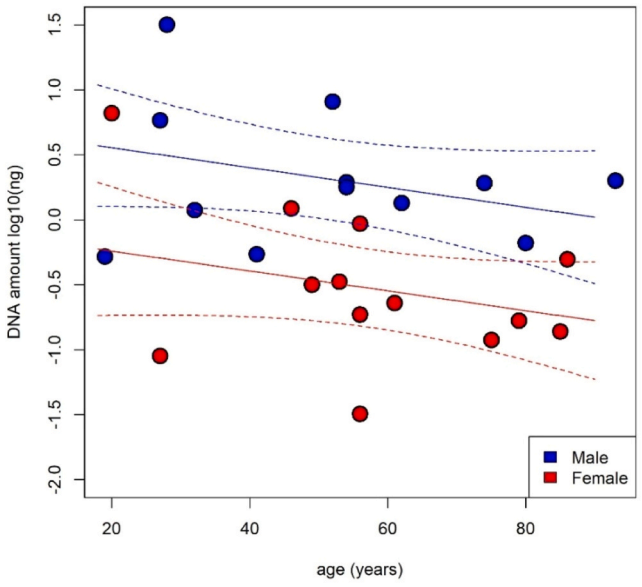

Fig. 4. Scatterplot of the log 10 total self-DNA across age for males (blue) and females (red). The solid line shows the regression function and dashed lines show plus or minus two standard errors.

suggests that the high shedder's partner possibly picked up this DNA from contacts made with surfaces and items from the shared living environment [43,44].

No significant differences were observed between the quantities of DNA deposits from participants who washed (or sanitised) their hands at the different time points (Fig. 5). Previously, recent handwashing was shown to significantly diminish touch DNA deposits [18,28,45] with some studies also suggesting that this effect is only relevant to higher shedders [26]. Equally, longer time frames of 1 h produced results consistent with this study [18], likely from DNA re-accumulation through both intrinsic and extrinsic processes.

No significant differences in DNA deposits were observed between the dominant and non-dominant hands, the hand size, residential

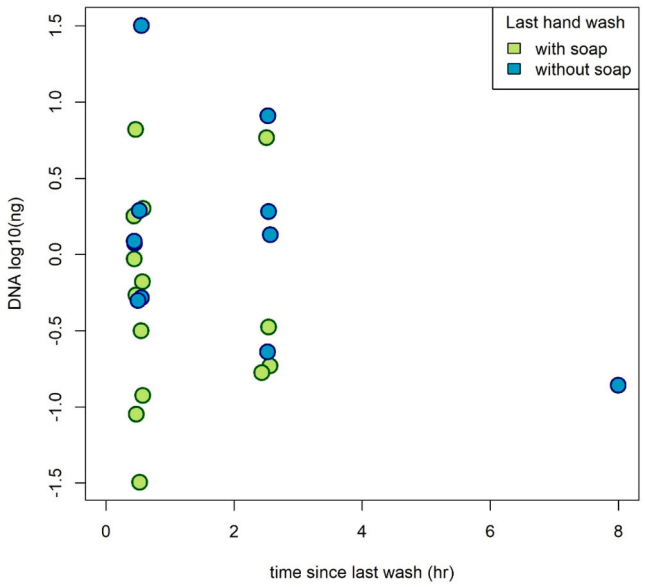

Fig. 5. Plot of log 10 total DNA amounts at different time frames post self-reported handwashing (total n = 25, with n = 14 having their most recent wash using soap and n = 11 without soap. A number of responses for last hand wash without soap were "Can't remember". In all these instances the responders had a documented time for the last wash with soap. Thus, it was assumed that their last hand wash was with soap (which they could remember the time of). Where both with and without soap handwashes occurred during the same time range (e.g., 2–3 h), these samples were added to the "without soap" group. Each point represents an individual.

location (remote, rural, suburban or urban), workplace (indoors and outdoors), use of moisturiser, recent eating or showering (<3 h and greater) (Supplementary Data 1). Self-reported face touching within an hour of sampling also did not produce significant differences in the deposition of DNA. However, face touching can be a subconscious action, rendering participants unaware of the contacts [46]. No correlation was found between quantities of non-self DNA and the number of co-habitants, the frequency of sharing a bed with someone, or number of people that share a bathroom. Interestingly, significantly more non-self DNA (p = 0.046) was detected on the hands of the individuals who worked indoors (mostly healthcare and education workers) than outdoors (mostly farmers; n = 7).

For 11 participants, there were multiple unknown donors detected in the fingernail and tube samples (Supplementary Data 1). Based on the mixture-to-mixture LRs (range of 9×10^{-01} to 4.5×10^{1}), no common unknown donor's DNA was detected for 10 participants (all inclusionary LRs of $<3.5 \times 10^{0}$). For a single participant (Participant 2I) a possible common, low level, contributor was detected between the left- and right-hand tube samples (LR = 4.5×10^{1}).

3.3. Shedder assessments

Our data-specific distributions, one each for the cell counting and both DNA methods, were utilised for shedder categorisation using methods outlined in Cahill et al., [44]. The category bounds for low, intermediate and high shedders were created using the mean value plus or minus one standard deviation (Supplementary Data 3 for all dataset distributions). Individuals' left and right deposits were combined and averaged for analysis.

The choice of self-DNA yields for shedder classification was based on the practicality of this approach due to its objective and quantifiable nature. Allele counts, relative fluorescent unit (RFU) values, and mixture proportions are directly related to DNA yield. Although non-self DNA detection information was not explicitly used, it was indirectly accounted for in the DNA yield data by considering only the DNA quantities attributed to self-DNA, as determined from STRmix mixture proportions (total DNA yields and self and non-self DNA yields based on mixture proportions can be found in Supplementary data 1).

3.3.1. DNA deposits between fingernails and held tubes

For DNA shedder classifications, the data was log10 transformed. For the tube deposition method, the 25 participants were categorised as low (16 %), intermediate (68 %) and high shedders (16 %), highlighting the high prevalence of intermediate shedders in a population.

Weak positive correlation was detected between DNA amounts found under fingernails and on the tube contacted by the same individuals (Spearman's correlation p = 0.022; Fig. 6). The self-DNA fingernail distribution was used to assign all participants into three shedder categories (Supplementary Data 1) and compared to the shedder assignment using the tube DNA method. The majority of participants (64 %) maintained the same shedder category. For the remaining participants (36 %), roughly equal numbers either increased (56 %) or decreased (44 %) by one category. A single participant has changed by two categories, dropping from high to low shedder.

The disparities observed in DNA shedding between fingernails and hand-held tubes can be attributed to complex factors underlying shedder categorisation. While the precise mechanisms responsible for individual variations in DNA shedding are not yet fully understood, several key factors are suspected to play significant roles [32]. These factors can be broadly categorised into biological, behavioural, and environmental influences, each contributing to the unique shedding profile of an individual. Behavioural factors encompass personal hygiene habits, occupation and daily activities, frequency of touching face or hair, and habits such as nail-biting or cuticle picking. The interplay of these factors may contribute to the differences observed between fingernail and hand-held tube samples. For instance, certain behaviours can significantly impact DNA accumulation under the fingernails. Frequent scratching can lead to increased accumulation of skin cells and DNA under the nails. Conversely, frequent hand washing generally reduces overall DNA on hands and may increase DNA concentration under nails by pushing loose cells into the subungual space.

3.3.2. Estimates of cell counts

The estimated total cell counts (entire finger grids) ranged from 506

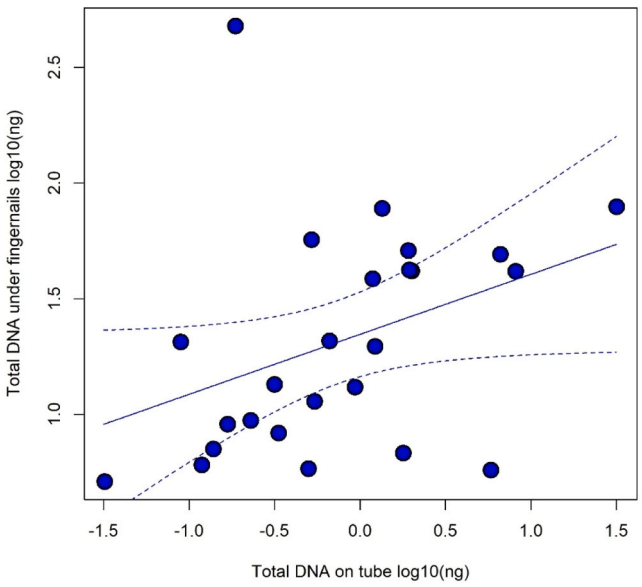

Fig. 6. Scatterplot of the log 10 total self-DNA under fingernails and on the handheld tubes. The solid line shows the regression function and dashed lines show plus or minus two standard errors.

to 14,334 (av. 3032 cells per print) (Supplementary Data 3). The majority of participants deposited between 1500 and 7500 cells (Fig. 7). These cell numbers were higher than cell counts reported previously where thumb and index prints yielded an average of 2399 and 816 cells, respectively [31]. However, there was a small number of individuals tested in that study (n = 6) reducing robustness of results. No significant difference was observed between cell numbers from dominant and non-dominant hands.

The cell number shedder categorisation was determined by assessing each cell count's position within a distribution. For the entire finger grid method, the majority of participants were classified as intermediate shedders (64.3 %). The second most common category in this study was low (21.4 %) followed by high shedders (14.3 %). These values align with the expectation of a normal population given that these categories are based on a normal distribution with boundaries at the mean plus or minus one standard deviation (thereby sectioning the distribution into 16 %, 68 % and 16 % of its area). Similar to present results, several studies found that intermediate shedders are the most prevalent category in the population [18,28,36,47]. Assessment of the whole fingerprint deposits showed that, in general, the middle of the finger deposited the majority of cells, compared to the periphery. However, this may be due to differences in the pressure and contact area that a finger makes during contact (heatmaps of all prints are in Supplementary Data 3).

There was a strong correlation between the 5x and 3x most populous grid cell methods [23,31](Spearman's Rank Correlation coefficient of 0.998). Further, comparison of the selected grid square (3x and 5x) methods to the entire print also showed strong correlation (Spearman's Rank Correlation coefficient of 0.860) (Fig. 8). The use of the most populated grid squares for shedder categorisation has been described previously [23] and has the time saving benefit, if shown to be comparable to the entire print method.

Shedder distributions were created for each of the three cell counting methods to assess possible classification differences of the same individuals attributed to method selection (Supplementary Data 3). Single category classification shifts were observed in 33 % (n = 14) of the participants tested when 3x and 5x grid square methods were compared to the entire finger grid method (Fig. 9). Not surprisingly, in the majority of these instances, 3x and 5x grid square methods resulted in higher shedder classification (i.e. from low to intermediate) and intermediate shedders were most consistent between the three classifications. Differences in classification, based on cell counting method selection (entire finger grid method vs 3 and 5 grids), have been described previously [31] indicating that the most representative, entire finger, method should be used to increase classification accuracy. In Fig. 9 we also show the change in shedding propensity in a continuous sense.

3.3.3. DNA results vs. estimates of cell counts

Comparison between entire finger grid estimated cell count and fingernail methods showed a large number of shedder changes. Only 40 % of participants maintained the same shedder category. No trends were noted where changes were observed, with 53 % being assigned lower and 47 % higher category. The majority of changes were by a single category (93 %). These findings suggest that fingernails and grid cell counts cannot be used interchangeably during shedder categorisation. These results support the findings of Johannessen et al., [27] that compared shedder categorisation achieved with cell counts and DNA depositions, finding only weak correlation between the two methods.

A positive correlation was observed between the DNA amounts deposited on tubes and grid cell count numbers (Spearman's correlation of 0.615; p- value <0.001). A weak to moderate relationship was noted between DNA amounts and cell numbers (Pearson's correlation coefficient of 0.42) (Fig. 10). Kanokwongnuwut et al., [48] also observed a linear relationship between DNA quantities and cell counts, indicating that cell counts can serve as predictors of DNA yields. However, a study by Cook et al., [49] investigated the use of Diamond dye™ in operation context and noted that some surfaces exhibit background fluorescence or nonspecific staining that made those surfaces unsuitable for DD staining. While a study by Madden et al., [50] showed that even though DD staining can be used to predict DNA yield, the predictive power decreased from controlled conditions (saliva deposits) to semi-controlled (finger marks) and uncontrolled (clothing) conditions. The likely degraded state of the DNA within corneocytes was proposed as a significant factor for these results. Furthermore, the DD technique, at the magnification used in that, as well as in the present investigation, is insufficient to visualise and quantify any cell free DNA that may have been present. This cell free DNA would, however, have been quantified during the quantification stage of the samples that underwent DNA processing. Unlike other studies [27,31,50], the results of this study indicate that cell counting can be used to predict DNA amounts (p = 0.002; Pearson's Correlation co-efficient) and that either method yields similar percentiles in shedder categorisation distributions.

It should be noted that while our choice of the glass slide surface (for DD shedder test) and plastic tube (for the DNA handheld test) was meant to reflect the most common surfaces used in the published shedder tests, it is well known that different surfaces can affect both cellular and cell-free DNA adhesion [51,52]and this may have affected the types of results that were generated in this study.

Shedder status remained unchanged between entire finger grid cell count and DNA tube method for 52 % of participants (Fig. 11), while 48 % of participants changed by one category. None of the participants had a two-category change. In general, continuous measurement approaches outperform categorical methods. Categories require thresholds

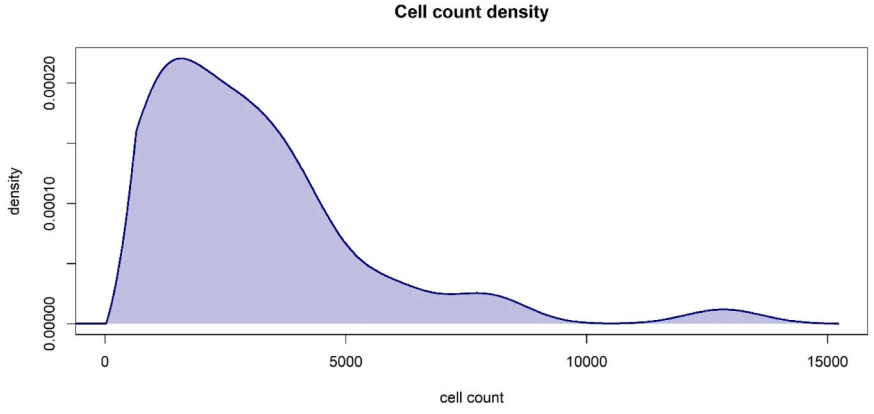

Fig. 7. Plot showing the distribution of total cell counts for the 42 depositors (participants and others) using the average of the combined cell counts of the left and right deposits per participant.

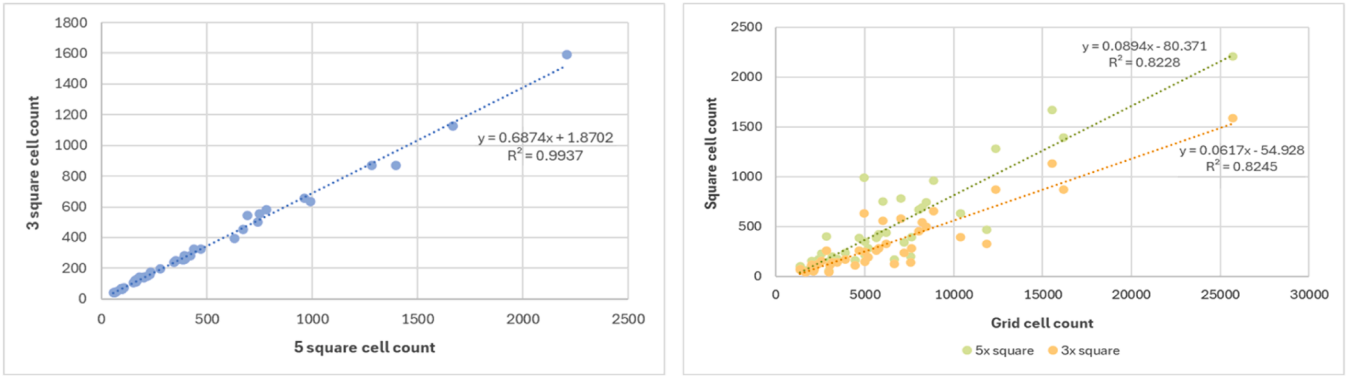

Fig. 8. Scatterplots showing the relationship between different cell count methods: 3x vs. 5x grid square methods on the left and entire print (grid cell method) vs selected grid square methods on the right.

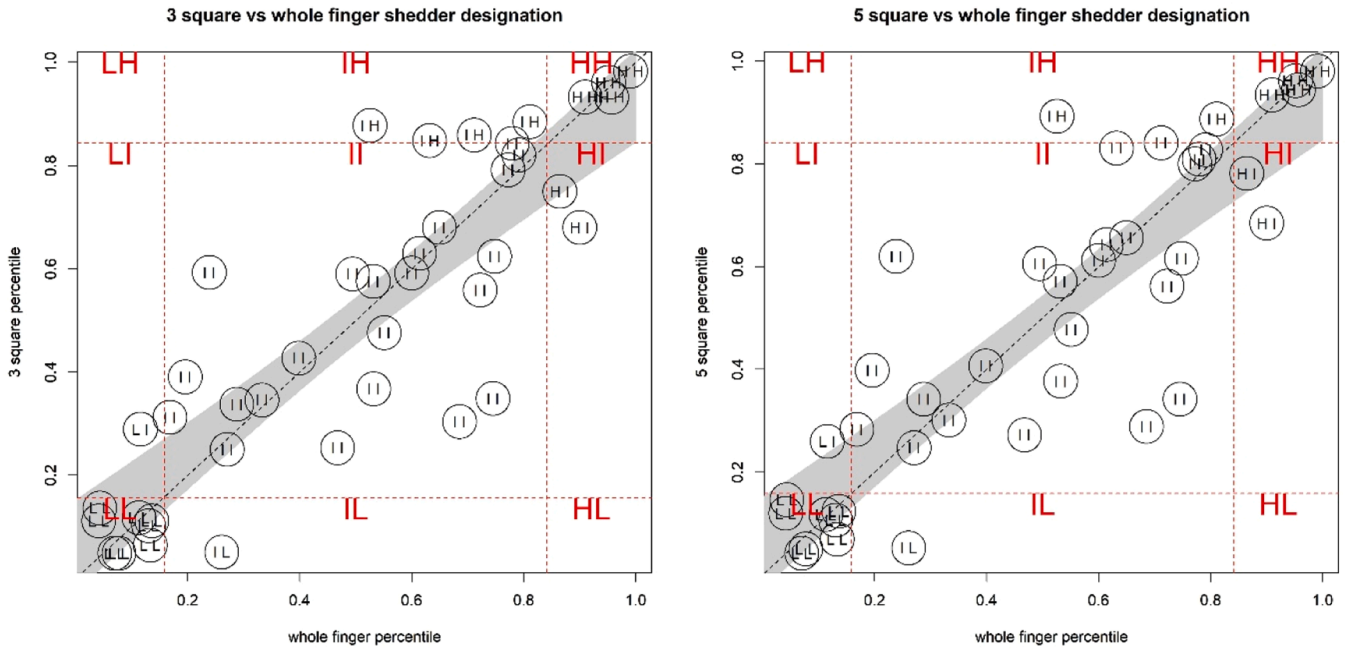

Fig. 9. Confusion matrices comparing different shedder status classifications between the 3x (left) and 5x(right) and entire finger grid cell counting methods. The x-axis represents the percentile that the individuals fall on the shedder distribution modelled on the whole finger and the y- axis shows the percentile that the individuals fall on the shedder distribution modelled on the 3 or 5 grid square methods. Red dashed lines represent the boundaries that demarcate low, intermediate and high shedder classifications (with the red lettering designating the classifications in the whole finger and grid square methods). The letters 'L', 'I' and 'H' represent Low, Intermediate and High shedder classifications. The grey polygon represents the regression of cell count against DNA amount plus or minus two standard errors.

and thresholds suffer from the 'falling off the cliff' effect. Further, several studies noted that intermedium shedders can show more variability, occasionally switching their shedder category [18,26,27], providing further support for the use of continuous categorisation methods. It should be noted that although cell count methods benefit from the ease of their use, they cannot account for any contribution of cell-free DNA [29] as this is not visible using the magnification used in this study, nor do they allow the separation of the deposits into self- and non-self contributions.

4. Conclusions

This study provides useful information on the prevalence of self- and non-self DNA under fingernails, supplementing previous studies and confirming that, in the absence of sexual or forceful physical activities, that may contribute sources of biological material containing abundant levels of DNA such as vaginal fluid or blood [3], non-self DNA is usually found as a minor contributor in less than half of all fingernail samples and 72 % of tube samples. Participants' partners were the most common source of non-self DNA. Further, datasets were collected on the quality and quantity of DNA deposits, and cell counts, following unrestricted everyday activities reflective of general population. We anticipate that these datasets will serve as valuable resource for activity level evaluations and encourage other investigators to contribute to the growing data collective. Here we have focused on the quantities and origins of DNA under fingernails and after direct deposits to tubes during everyday activities, however, similar information from other body areas is currently lacking [53,54]. Data on the prevalence of self-and non-self DNA in everyday situations will provide better contextualisation of results, accounting for individuals' recent activities. Further studies focussed on the timelines and actions associated with the acquisition and retention of non-self DNA on hands versus fingernails would be welcome.

Furthermore, the assessments of three shedder testing methods—cell

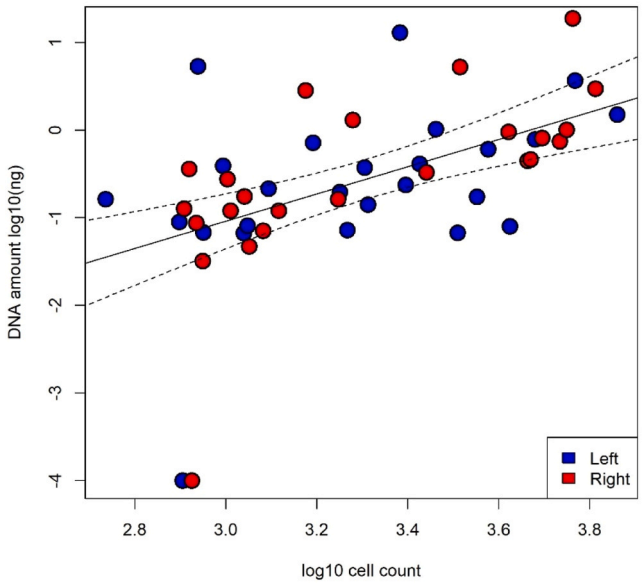

Fig. 10. Scatterplot of the relationship between log10 total DNA amount deposits on tubes and entire finger grid cell counts (also on log10 scale) for each participant (left and right hands shown separately). The solid line shows the regression function and dashed lines show the plus or minus two standard errors. Value of 0 are plotted at −4.

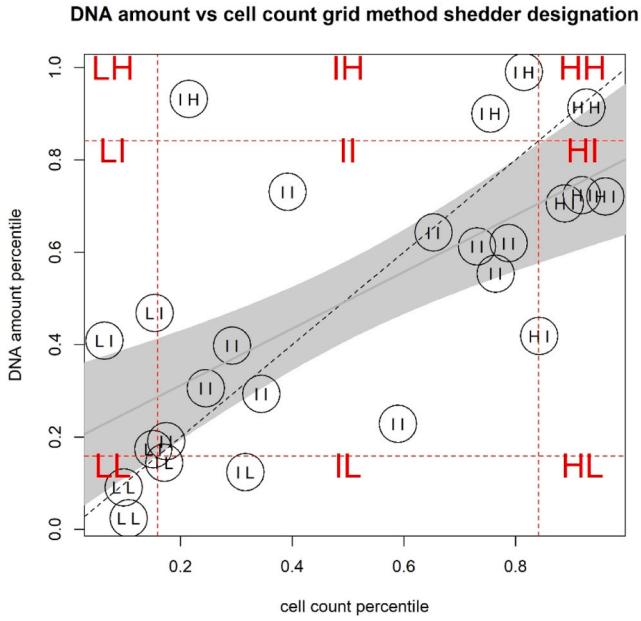

Fig. 11. Confusion matrix comparing differences in shedder categorisaton between DNA deposited on tubes and entire finger grid cell count methods. The x-axis represents the percentile that the individuals fall on the shedder distribution modelled on the whole finger cell count and the y- axis shows the percentile that the individuals fall on the shedder distribution modelled on the DNA amount. Red dashed lines represent the boundaries that demarcate low, intermediate and high shedder classifications (with the red lettering designating the classifications in the whole finger and grid square methods). The letters 'L', 'I' and 'H' represent Low, Intermediate and High shedder classifications. The grey polygon represents the regression of cell count against DNA amount plus or minus two standard errors.

counts, fingernail swabs, and direct contact DNA samples (tube holding)—have demonstrated that these approaches cannot be used interchangeably for shedder assessment. This finding underscores the need for caution when utilising or combining data from different studies. The shedder study by Schwender et al., [55] tested two different DNA sample processing workflows highlighting how DNA methodologies can affect shedder classification. Further, a recent study by Taylor et al., [56], acknowledging the influences that DNA processing methodologies may have on activity level evaluations, proposed a corrective framework to allow the use of inter-laboratory data. Additionally, even when the same testing methodology is used, variation in shedder classification methods can result in differences in shedder designations [20]. Given the diversity of methods currently in use, there is a pressing need to establish a single, widely accepted method for shedder assignment moving forward. This standardisation would allow for the expansion of a reliable database of known data, thereby improving the accuracy of DNA evaluations. However, crucial questions remain unanswered: what are the primary objectives of shedder classification, and which tests and means of categorisation most effectively address key aspects? For example, which tests most faithfully represent contacts and deposits that a person of interest may make at a crime scene. This question appears most relevant to activity level evaluation and thus requires our attention. Such testing would need to be easily integrated into standard investigative processes where, hypothetically, a police officer might one day perform a shedder test alongside routine collection of DNA reference samples and fingerprints. Looking at the scope of the data available today, it is clear that further work is needed before any realistic recommendations can be made. It is not our intent to make this determination in the present study, rather we aim to highlight the issue at hand and encourage the forensic community to participate in the conversation.

CRediT authorship contribution statement

Kahli Murton: Writing – review & editing, Investigation, Formal analysis, Data curation, Conceptualization. **Duncan Taylor:** Writing – review & editing, Supervision, Project administration, Methodology, Formal analysis, Conceptualization. **van Oorschot Roland R.H.A.:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization. **Adrian Linacre:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Conceptualization. **Mariya Goray:** Writing – original draft, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The author is an Editorial Board Member/Editor-in-Chief/Associate Editor/Guest Editor for Forensic Science International: Genetics and was not involved in the editorial review or the decision to publish this article.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.fsigen.2025.103367.

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