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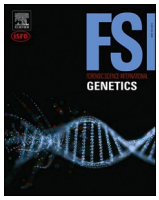

Where did it go? A study of DNA transfer in a social setting

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ABSTRACT

The sensitivity of DNA analysis has progressed to the point that trace levels of DNA, originating from only a few cells, can generate informative profiles. This means that virtually any item or surface can be sampled with a reasonable chance of obtaining a DNA profile. As the presence of DNA does not suggest how it was deposited, questions are often raised as to how the DNA came to be at a particular location and the activity that led to its deposition. Therefore, understanding different modes of DNA deposition, reflective of realistic forensic casework situations, is critical for proper evaluation of DNA results in court. This study aimed to follow the movements of DNA to and from individuals and common household surfaces in a residential premises, while socially interacting. This took place over an hour and involved four participants, with known shedder status, designated as visitors (a male and a female) and hosts (a male and a female), who engaged in the activity of playing a board game while being served food. During the study, the participants were instructed to use the toilet on a single occasion to assess the transfer of DNA to new and unused underwear that was provided. All contacts made by the participants in the dining room and kitchen were video recorded to follow the movements of DNA. Samples were collected based on the history of contact, which included hands, fingernails and penile swabs. Direct contacts resulted in detectable transfer (LR > 1) in 87 % (87/100) of the non-intimate samples and clothing. For surfaces touched by multiple participants, DNA from the person who made the last contact was not always detectable. The duration and number of contacts did not significantly affect the detection of the person contacting the item. On the other hand, presence of background DNA and participant's shedder status appear to play an important role. Further, unknown contributors were detected in the majority of samples. Finally, indirect transfer was observed on a number of occasions including co-habiting partners of guests who were not present at the study location. The results of this study may assist with decision making for exhibit selection or targeting areas for sampling within the home environment. Our findings can also be used in conjunction with previous literature to develop activity-level evaluations in such situations where the source of the DNA is conceded, but the mode of deposition is disputed.

1. Introduction

Due to the trace nature of many of the biological samples submitted to forensic services [1–6], challenges to this type of evidence in court are shifting from questioning identity towards questioning activities that lead to its deposition [7,8]. Evaluating the evidence given activity level considerations is based on assignment of probabilities for DNA transfer, persistence, prevalence and recovery (TPPR). To make these assignments requires data availability, relevant to the interpretation at hand [9–16].

Studies into DNA- TPPR issues can be categorised as controlled [17–19], semi-controlled [20–27] or uncontrolled [28], and each provide information that is useful in a different context. Controlled studies will attempt to limit factors and measure as many aspects of the action as possible and are useful to gain understanding about the fundamental factors affecting observations arising from an action. Semi-controlled studies are often conducted in uncontrolled environments (such as a house or office building) but seek to provide some control over the types of actions that will occur during the experiment. Semi-controlled studies are useful when we seek information about how actions may occur in the

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real world. Uncontrolled studies do not seek to control any aspects of the world and are often studies that provide a DNA snapshot of the world. They may include environmental sampling of a laboratory, sampling of people's hands after a party, or sampling of objects in a home without any instruction and limited knowledge on what actions occurred leading up to the samples being taken. Uncontrolled studies are useful to show the expected DNA amounts from the variation that can exist in the actions performed and how the actions are carried out in the real world. Activity Level Evaluations (ALE) allow scientific evaluations of findings given two opposing propositions: the prosecution's position and the defence's position at the activity level in the Hierarchy of Propositions [29,30]. To evaluate activity level propositions, DNA-TPPR data is vital [31]. This research aims to add to the growing body of knowledge on DNA-TPPR by providing a set of data that may be useful in ALE's.

DNA transfer has been investigated under various conditions in controlled environments in several studies, however limited studies exist on more realistic scenarios and uncontrolled conditions [28,31]. A number of recent studies investigated the movements of DNA in an office space showing that DNA can transfer to and from non-occupants of the office and was dependant on the nature of contact and surfaces involved [28]. However, a common crime scene location [7,8,32], a residential house, has been a subject of a limited number of investigations. Recently, Reither et al. [33] investigated prevalence of human DNA on different flooring surfaces in an occupied house finding detectable DNA in 97 % of the samples and further showing that DNA on the floors can be readily transferred, including to and from worn clothing [34]. While these studies provide useful information, many common household social situations still require investigation.

In this study, movement of DNA in a social setting was investigated. The two owners of the house hosted a social gathering for two visitors that have not been to the premises nor knew the hosts prior to the experiment. Limited instructions were given to the participants with the majority of contacts being unscripted, with the exception of no direct physical contact and restricted access to the kitchen. These interactions were recorded with seven video cameras. One of the instructions required the participants to attend the toilet in the middle of the experiment to assess DNA transfer to surfaces within this area and to new pairs of underpants worn by the participants on the morning of the experiment. DNA samples were collected based on the contact histories, ascertained from the review of video recordings and participant instructions, to include items and surfaces not touched by anyone to surfaces touched by all four participants as well as personal items and body samples such as clothing, hands, self-sampled penile swabs and pubic combings. The DNA results from these samples were analysed in light of the contact histories of each item/surface, further information determined from participant questionnaires relating to basic demographics, personal habits and individual's shedder status.

2. Material and methods

2.1. Experimental design

Four individuals participated in a social interaction of playing a board game while eating and drinking. Fig. 1 shows the timeline of events in the experiment.

The interaction took place in the residential premises and lasted for approximately 1 hour. Two participants, a female and a male (the visitors; not known to each other), attended the host's (a female and a male) house. Hosts last cleaned the premises 2 weeks prior to the experiment. The visitors had never attended this residential premises nor knew the hosts. Participant interactions during the experiment were unscripted with the exception of a small set of instruction (Supplementary Data 1A). The participants were instructed to wear supplied, new, unused underpants and clean clothes on the morning of the experiment. Visitors were instructed to enter the premises separately, where they were guided to the dining room by one of the hosts. All participants were instructed not to touch each other during the experiment. The participants were not assigned seats and took a seat at random around a plastic fold-out table (permanent table used by the participants). The male host had set up the newly purchased board game (Pressman Toys – Pop 'N' Hop) on the table 30 minutes prior to the experiment. During the 10-to-40-minute period of the experiment, the participants played the game while consuming food and beverages (individual cup of tea and plate with cake) served by the female host. Forty minutes into the experiment, the participants were instructed to go to the toilet in the following order: female visitor, male visitor, female host and male host. Female participants were instructed to stand and sit three times, while touching the toilet roll to mimic what may be normally contacted during toileting. The males were instructed to stand and simulate urination, contacting their underwear and penis as they normally would. Following this, all participants flushed the toilet and washed their hands with liquid hand soap and hand towel (~38×66 cm^2) provided by the host (used for 2 days prior). During the period of bathroom breaks, the female host removed the cups, plates and cutlery from the table and placed them in the kitchen. On return to the dining room, participants collected cleaned glasses provided by the female host (collected from cleaned box; glass surface only touched by the individual participants) and poured themselves a drink from a shared jug while eating lollies from a cleaned jar and talking. The jug and the jar were brought in by the female host after the toilet break. At the conclusion of the experiment limited number of samples were collected from the participants and the experimental house. The participants remained in their seats while personal samples were taken from the two visitors in the following order: female visitor followed by the male visitor. Both visitors departed separately immediately after sampling (approx. 35 minutes post social interaction), and the hosts stood up and were sampled. Thus, for several items (such as

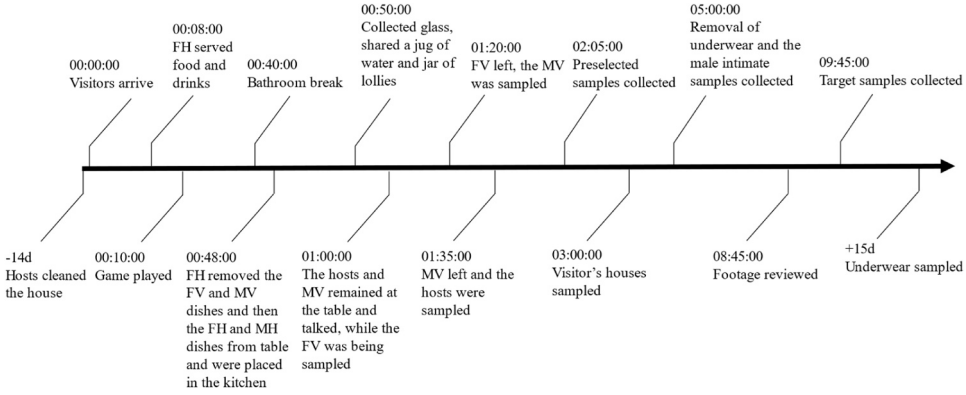

Fig. 1. The timeline of the events that occurred leading up to, during and post experiment. Individuals involved are FH (female host), MH (male host), FV (female visitor) and MV (male visitor).

chairs) duration of contact was longer than 1 hour (Supplementary data 4). Hands and fingernail samples of the hosts only, and clothing surfaces from all participants were sampled at this time (Supplementary data 1A). The visitors were instructed to drive directly home and continue with their daily routine. The hosts were instructed to wait outside while the initial predetermined samples were collected at which time they were allowed to return home but not allowed to go into the dining room or the kitchen until all samples were collected. Five hours following the experiment the participants removed the supplied underpants and packaged it into the provided new yellow envelopes, which were sampled approximately 2 weeks post experiment. At this time, male participants were additionally instructed to self-collect penile samples and pubic combings (Supplementary data 1B). Questionnaire responses on basic demographics and activities performed on the day of the experiment were also collected (Supplementary data 2A and B). This study was conducted under the Flinders University Human Ethics Approval (4915).

2.2. Experiment preparation

A set of new glasses, a jar and a vase were cleaned prior to the experiment using 1 % hypochlorite and sterile water. These items were then exposed to UV light for 20 minutes. The four pairs of underwear were covered in plastic when purchased and were not sterilised further. No other items were cleaned prior to the experiments. Control samples were taken from each glass, the jug and the vase as well as the glass plates used for shedder testing to assess the success of the cleaning. Additionally, four background samples were collected prior to the experiment; one from each side of the table where the participants did not sit (~10x10cm^2), one from the drawer and one from the cupboard in the kitchen.

Reference samples were collected from the hosts and visitors, as well as the close associates of the visitors (two co-residents of the male visitor and an inhabiting partner of the female visitor). Neither the visitors nor their close associates had previously visited the experimental house. A reference sample was also collected from the researcher.

2.3. Video recordings

Seven cameras, four positioned around the dining room table and three placed in the kitchen were used to record all contacts made by the participants (with the exception of the toilet visits that were not recorded) (Fig. 2). All cameras were positioned to film from different angles to ensure all movements and contacts in these zones could be captured and recorded. All participant hand contacts were reviewed by the researcher after the experiment and information documented regarding the surfaces touched, duration and number contact (separately for left and right hands). The recordings were also used to determine the surfaces/items and specific locations or areas on these surfaces to target.

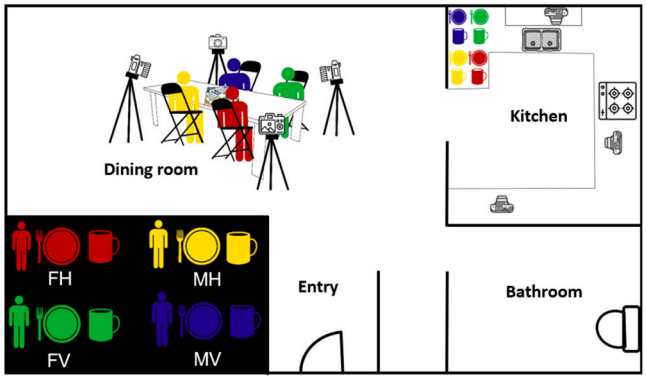

Fig. 2. Camera and participant placement in the house. Individuals involved are FH (female host), MH (male host), FV (female visitor) and MV (male visitor).

2.4. Shedder status determination

Participant shedder status deposits were collected as per Goray et al. [35] with some modification. Each participant was asked to place their dominant hand onto a clean glass plate for 10 seconds with firm-moderate pressure; 30 minutes after hand washing. Deposits were made on three separate days, apart from the female host, where two of the three samples were collected on the same day 5.5 hours apart. All samples were processed for DNA as per Section 2.5.

The amount of DNA on individuals' hands is one way in which shedder status could be assigned. Within a population, DNA on hands is likely to be distributed in a continuous way, rather than being present in distinct high, intermediate and low shedder groups. If we want to classify an individual as being a high, intermediate or low shedder then the population level of DNA on hands can be modelled, and this distribution can be broken into quantiles that designate the boundary of shedder classification. There are an infinite number of ways in which a continuous distribution can be discretised, and all are equally arbitrary. We choose to model log10 transformed total DNA amounts using a normal distribution. The DNA amounts were transformed so that it approximately adhered to a normal distribution.

The 10 individuals from Goray et al. [35], who had both hands sampled (individually) 12 times were used. For each individual, the 24 hand samples (left and right) were averaged and the log10 of the average DNA amounts modelled with a normal distribution. Thresholds for low/intermediate and intermediate/high shedders were used to zone the normal distribution and were set at the mean minus or plus one standard deviation. The shedder status of the participants in the study were then determined by which zone they fell within.

2.5. Sample collection and processing

Samples were selected in two ways: preselected samples (determined during experiment design) and video recording targeted samples. The preselected samples were known to be touched by participants during the social interaction based on pre-experimental instructions including game pieces, cutlery, body samples and underpants (Supplementary data 4). Video targeted samples focussed on the unscripted actions of participants which may vary with social behaviour. Both preselected and targeted samples fell into one of these categories: touched by all participants, touched by three participants, touched by two participants, touched by one participant, and not touched during the experiment.

The following preselected samples were collected immediately after the experiment: the host's and the visitor's forearms/external sleeves (that contacted the table; note: male visitor wore long sleeve top and sample was collected from the clothing; for the rest of the participants forearm skin samples were taken), the back of their pants (that had contact with their seat), the car keys, game pieces, furniture, cutlery and toilet surfaces as well as the hands and fingernails of the hosts (see Supplementary data 4 for the full list of pre- selected and targeted samples). Immediately after the initial sampling of the experimental house, additional samples were collected from the visitor's homes. The inner front door handle, external car door handle, car seat, car steering wheel and three surfaces/items participants contacted directly after returning home were sampled. Hand and fingernail samples were collected from the visitors at that time.

The video footage was then viewed to determine further surfaces for target sampling. The subsequently targeted samples were all located in the kitchen and consisted of cupboard and drawer handles. The supplied underwear was collected 5 hours after the experiment and the following samples were later taken: gusset, left internal and external wing, right internal and external wing, and internal and external waistband. At the time of the underpants removal, the male participants were also

instructed to self-collect penile swabs and pubic comb samples (Supplementary data 1B).

A total of 128 experimental samples were collected using the wet and dry double swabbing technique [36] (SARSTEDT DNA-free 'Forensic Swab L'). DNA was extracted using DNA IQ™ (Promega; in 60 μl volume), quantified using Quantifiler Trio™ (Applied Biosystems; LOQ 5 pg/μl; 2 μl volume) and amplified using PowerPlex® 21 kit (Promega) up to a maximum template of 0.5 ng in a 25 μl reaction volume (30 cycles). All samples were submitted to the amplification step, independent of their detected DNA concentration. The female participants' hands, fingernails, underwear and the reference samples of the male participants and the female visitor's male partner were further amplified using Yfiler™ Plus kit (Thermofisher Scientific) up to a maximum template of 0.5 ng in 25 μl reaction volume (30 cycles), typed with 3500 genetic analyser (Thermofisher Scientific; 1.2kv/24 sec and 1.5kv/24 sec for PowerPlex®21 and YFiler™Plus, respectively) and GeneMapper ID-X (v 1.6; Thermofisher Scientific, Waltham, MA, USA).

2.6. Data analysis

The total DNA amount (ng) for each sample was estimated by multiplying sample DNA concentration (ng/μL) by the elution volume (60 μL). The minimum number of contributors was manually determined utilising the maximum allele count (MAC) method [37–40] in combination with peak height balance. STRmix™ v2.9 (ESR and FSSA) was used to determine mixture proportions, contributor RFU, mixture to mixture comparisons and person of interest inclusions/exclusions. For POI number of alleles counts, homozygote alleles were counted twice if the allele in question was above the homozygote threshold of 2000RFU. For mixed DNA profiles STRmix mixture proportions were used to allocate RFU contributions.

Participants were determined to be a major contributor if they contributed 70 % or more of the total DNA and a majority contributor if they had contributed most of the DNA to the sample as determined by STRmix. The samples additionally processed with Yfiler™ Plus were analysed using YHRD (Y chromosome haplotype reference database) with Eurasian–European database (observed frequency in the database reported [41]). To determine if there was a relationship between the amounts of DNA recovered and the number and duration of contacts, a Kruskal-Wallis H test was performed ($p < 0.05$; IBM® SPSS® Statistics v28).

3. Results

3.1. Control and background samples and shedder categorisation

3.1.1. Control samples

Of the items cleaned and sampled prior to the social setting, three of the four glasses, the jar and the vase produced partial profiles with 2–4 alleles (0.007–0.03 ng total DNA; Supplementary data 4). Statistical analysis favoured exclusion of all participants with the exception of the female host (2/9 samples) and the researcher (3/9 samples) (all LRs < 100 in favour of inclusion). These alleles are likely a result of incomplete cleaning or low-level contamination from the environment. One of the three controls from the cleaned shedder glass plates also produced a partial profile (0 ng, 3 alleles) with low inclusionary support for the researcher (LR = 13).

3.1.2. Background samples

All four background samples (non-touched areas of kitchen table (end 1 and end 2), cupboard and drawer) produced two person mixtures (0.42–0.72 ng) with the male and female hosts included in all samples, while the visitors were excluded as contributors.

3.1.3. Shedder categorisation

The female and male hosts were categorised as low and intermediate and female and male visitors as low and high shedders respectively (Supplementary data 3; refer to Supplementary data 6 for discussion regarding number of samples per person). Fig. 3 shows the log 10 total DNA amounts for the participants and the log10 total DNA distribution sourced from the total DNA amounts published previously [35]. All the other profiling parameters that may be used for shedder classification for the four participants are provided in the Supplementary data 3.

3.2. DNA yields, number of contributors and profiling results

Of the 128 samples collected, one sample did not generate a DNA profile or quantifiable DNA (vase) and was not analysed further. This was a pre-determined untouched item as per participant instructions. The vase was in the vicinity of the participants for the duration of the interaction. Other studies [42–44] have observed that speaking can spread DNA to surfaces within close proximity, but this was not observed here. Note that a control sample was taken from the vase producing a partial profile. Therefore, this initial sampling removed the surface DNA from prior contacts.

The total DNA amounts from the remaining items and surfaces (127 samples) ranged from 0.06 ng to 46 ng (av. of 3.5 ng) (Supplementary data 4; Fig. 4A-for average amounts of DNA detected for each category per participant). Note that the lower DNA amounts (below 0.3 ng) are below the limit of quantitation of Quantifiler Trio and so are subject to greater variability in the measured DNA amount. The number of contributors ranged from 1 to 4 (av. of 2). The majority, 60 % of profiles, were two-person mixtures, followed by single source profiles (26 % of samples), three person mixtures (12 % of profiles) and four-person mixtures (2 samples or 2 % of profiles). The two four person mixtures were generated from door handles to the toilet, in the experimental house, and the male visitors' home front door.

The samples with the lowest total DNA (<0.3 ng; 28 samples; Fig. 4B) showed that the majority of these samples (10/28) were from the female host (underpants and touched items such as fork) and (4/28) were used by the female visitor (underpants, fork, game piece and car door handle). The remaining samples (14/28) were from the male visitor (underpants, clothing and two touched items:). The only two non-clothing items with low quantification results for the male visitor were the game piece and the fork (0.12 ng and 0.24 ng, respectively). The game piece was contacted by the male visitor 29 times for a total duration of 1 minute and 53 seconds and the fork was contacted 6 times for 1 minute and 35 seconds. When compared to other participants these amounts were higher or the same as what was detected for two low shedders (0.06 ng for game piece and fork for the FH; 0.24 ng and 0.18 ng for game piece and fork of the FV). However, male host generated 3.1 ng and 4.9 ng from the game piece (contacted 48 times for 3 minutes 51 seconds) and the fork (5 contacts for 1 minute and 15 seconds). The male host has made the second greatest number of contacts during the interaction (see Section 3.3) possibly loading his hands with background DNA.

There were 65 samples with total DNA amounts in the range of 0.3–3 ng (Fig. 4B), 43 % of which were clothing and body samples. Samples that were associated with the intermediate and high shedders (male host and male visitor) accounted for 38 %, while samples associated with the two low shedders (female host and female visitor) accounted for 42 %, both showing similar results for this quantification range. The remaining 20 % related to samples taken from surfaces within the home (taps, handles, cupboards, toilet flush button, toilet seat and bathroom towel).

Of the 34 samples with the largest total DNA amounts (>3 ng; Fig. 4B) 59 % were collected from the intermediate and high shedders, 35 % were collected from the two low shedders, and the remaining 6 % related to non-personal items. Just over half of the samples in this category were collected from participant's clothing and body samples.

Participants were detected in 123 of the 127 samples. The four profiles, where all participants were excluded or favouring exclusion,

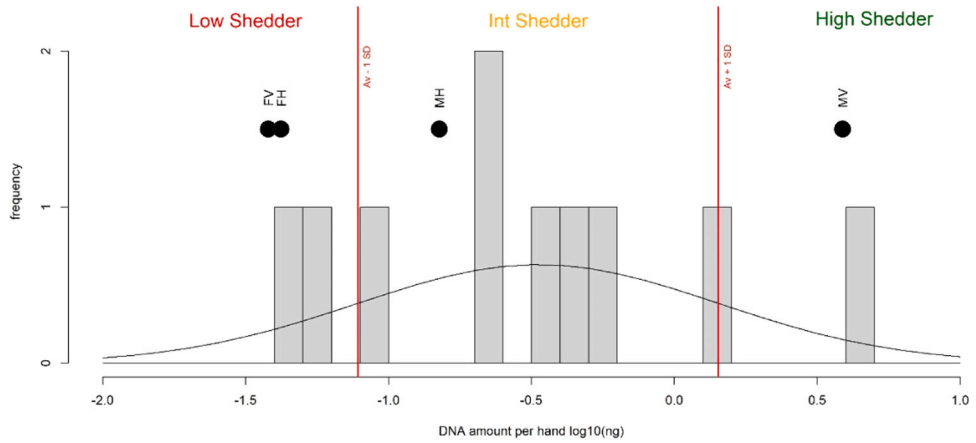

Fig. 3. The total DNA amounts [35] shown in the 24 observations from each individual where averaged and transformed to a log10 scale (grey bars). The data was modelled with a normal distribution (black line). Thresholds (red vertical lines) for low, intermediate and high shedders were set at the mean of the modelled distribution (-0.48) minus or plus one standard deviation (0.63). The four participants are plotted as points on the distribution (FV=female visitor; FH=female host; MH=male host and MV=male visitor). **FH=female host; MH=male host' FV=female visitor; MV=male visitor.*

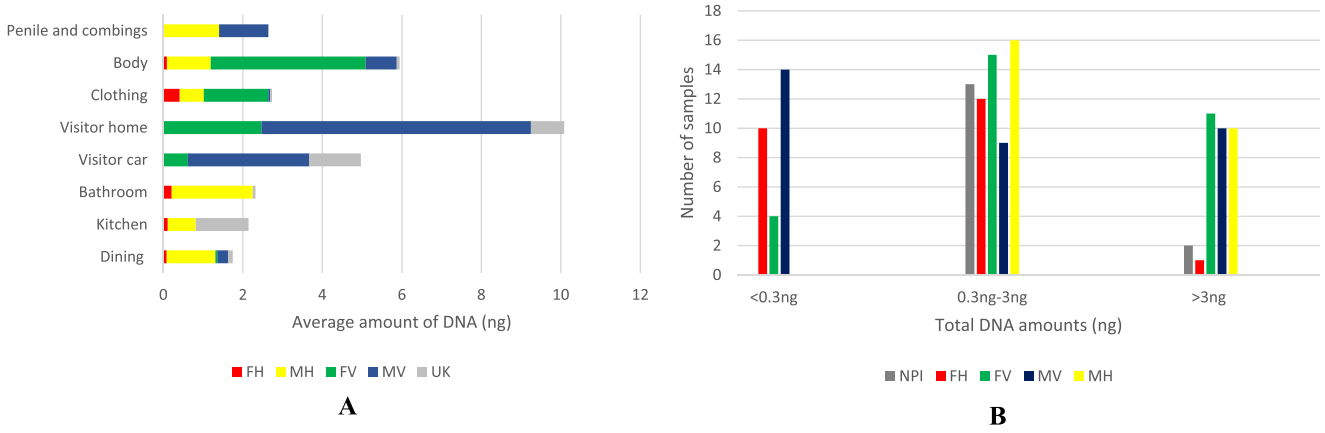

Fig. 4. A. Bar chart showing average DNA quantifications results taken from surfaces/items (separate for those sampled at host (separate for bathroom, kitchen and dining) and visitor houses and cars), intimate (penile swabs and combings), body (including fingernails, hand swabs and forearm samples where applicable) and clothing. The bars represent average amounts of DNA detected with each participant making up the bar- coloured red (FM), yellow (MH), green (FV), blue (MV) and grey (unknown). **FH=female host; MH=male host, FV=female visitor, MV=male visitor; UK=unknown.* B. DNA quantifications results for the 127 samples separated into samples with less than 0.3 ng of total DNA, samples with 0.3–3 ng of total DNA and samples with greater than 3 ng total DNA. Samples were further broken down based on samples that could be associated with a participant (e.g., clothing, body samples or personal forks etc.; red (FM), yellow (MH), green (FV) and blue (MV)) and non-personal items (grey NPI's) (e.g. jug, bathroom tap, dice etc). *The amounts breakdown is arbitrary but aims to reflect basic expectation of DNA profiling and trace sampling where optimal template amounts tend to be around 0.5–1 ng (encompassed in 0.3–1 ng bracket) and samples with less than 0.3 ng of DNA may produce sub-optimal results and samples with >3 ng of DNA are likely to have non-touch contributions.*

were all partial in nature and collected from male visitor's underpants (inner surface region; 5 alleles), male visitor's clothing (right sleeve; 16 alleles), toilet button (19 alleles; male visitor's house) and female host's left fingernails (9 alleles).

3.3. Number and duration of contacts

The total number of contacts (left and right hands for all surfaces/items excluding toilet visit, clothing and body touches) made during the experimental hour was 356 and 415 for male visitor and male host and 321 and 588 for female visitor and host respectively (Supplementary data 4). The total number of contacts made by each participant with the sampled surfaces (excluding body and clothing) ranged from 187 to 423. Fig. 5 shows the pattern of contacts on a timeline for some selected items that were present in the filmed interactions and whose DNA was detected in the samples collected from these items. A complete graph showing interaction with all surfaces/items, and the resultant DNA profiles, is available in Supplementary data 5.

The female host made most contacts with the sampled surfaces, as this participant was tasked with preparing and serving the food. However, of the 32 non-intimate items that this participant had touched, 44 % (14/32) resulted in exclusion or favouring exclusion. The majority of these surfaces were a short contact of less than a minute, with the exception of the mug, contacted 37 times for a total of 6 minutes, 45 seconds. The female host gave inclusionary support for the majority of household surfaces that are commonly used in the home. Notably, when detected, the female host was the minor contributor in 94 % of the non-intimate samples (17/18).

Similarly, female visitor was excluded from 38 % (10/26) of the surfaces that she touched and when detected was the minor contributor in 31 % (5/16) of the samples. While many contacts were less than a minute or unknown, for example for toilet samples, both chair seat and back, that the participant sat on for over 57 minutes, resulted in exclusion. The inclusions, likewise, were from surfaces that likely had background DNA from the female visitor (home iPad, home door etc).

The male visitor was detectable in 56 % (14/25) of the surfaces

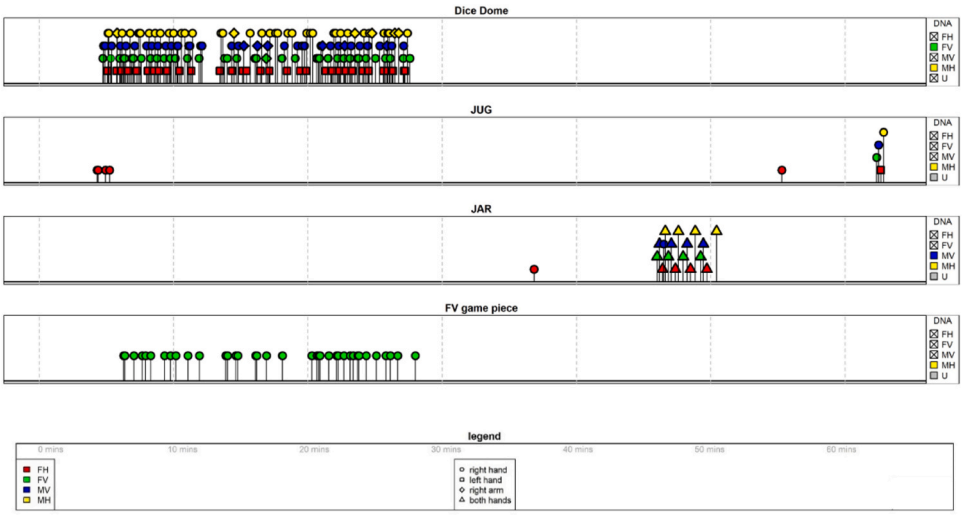

Fig. 5. Interactions between people and some selected surfaces/items during the social aspect of the experiment. The x-axis represents the time since the beginning of the experiment. The colours represent the individuals involved and the shape of the points represents the body part contacting the object (as provided in the legend). The position on the y-axis has no meaning, and points are separated on this dimension based on individual (to improve visual clarity only). On the right-hand side of each object's timeline, there is a list of all four individuals and 'U' which represents unknown DNA. In these right-hand panels, those individuals whose DNA has been detected on the item are coloured, and those whose DNA was not detected are marked with a cross and are uncoloured.

touched and when detected, this participant was the sole or the major contributor in 93 % (13/14) of the samples which is likely a reflection of his shedder status. For the number of surfaces, where this participant was excluded, the contact duration could not be recorded (toilet areas), and most of the other surfaces had brief contacts of less than a minute.

The male host was detected on 100 % (21/21) of surfaces touched and when detected was always either the sole or the major contributor. This may be a result of his shedder status and natural DNA accumulation within his own home. Notably, this participant touched his face, body and clothing approximately 55–110 more times (231 contacts) than the other three participants (176, 133 and 121 contacts for female host, female visitor and male visitor, respectively). It is possible the hands of male participant have been loaded with his DNA from touching his body and personal items. Jansson et al. [45] showed that "active" hands, that were allowed to contact surfaces, accumulated more DNA from other parts of the body and previously contacted surfaces than "inactive" hands, that were not allowed to touch anything.

The number of contacts made did not have a significant effect on the amount of DNA detected on a surface (p=0.113; Fig. 6); both when all items were analysed together and separately for each participant, however, background DNA may have impacted the interpretation. The average amount of DNA detected on surfaces touched less than 15 times was higher (0.22 ng; median 0.05 ng; SD 0.39 ng) than average amounts detected on surfaces touched 16–40 times (0.02 ng; median 0.01 ng; SD 0.04 ng) or more (44–153 contacts; 0.14 ng; median 0.03 ng; SD 0.26 ng). Similarly, duration of contact did not have a significant influence on the amounts of DNA detected (p=0.188), with an average of 0.38 ng detected after short contacts of less than a minute (median 0.18 ng; SD 0.45) compared to longer contacts of 1–5 minutes (av. 0.04 ng; median 0.012 ng; SD 0.05)), 5–25 minutes (av. 0.01 ng; median 0.01 ng; SD 0.01) or longer (25–76 minutes; av. 0.05 ng; median 0.04 ng; SD 0.06). Notably, in this study, the shorter duration and number of contact surfaces were, generally, with items such as cupboard, fridge and drawer handles, kettle and kitchen tap; all of which are likely to be in regular use. Conversely, surfaces that were touched more frequently and for longer duration were, for the majority, surfaces that were either new to the household such as game pieces, glasses and jar or were surfaces that are likely to be cleaned such as mugs and plates, thus removing background DNA.

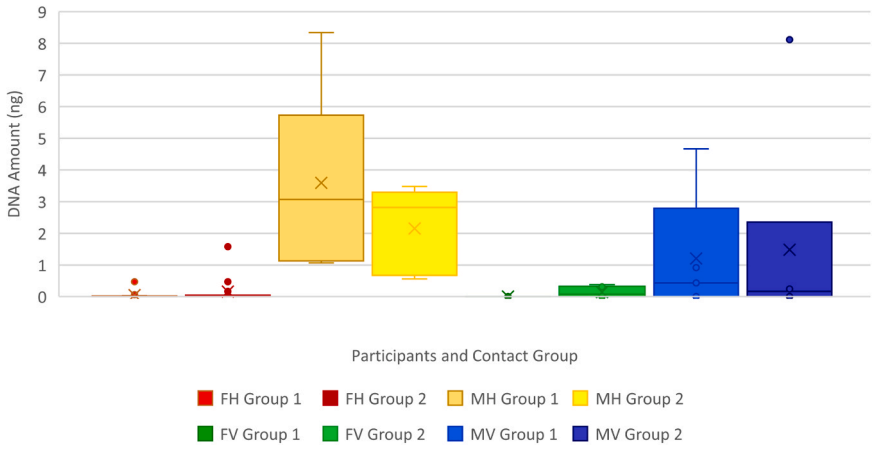

Fig. 6. Contributor DNA amounts in samples the participants had contacted separated into five or less contacts (Group 1) and more than five contacts (Group 2). **FH=female host; MH=male host, FV=female visitor, MV=male visitor; UK=unknown.*

3.4. Experimental home, visitor's home and visitor's car surfaces contacted by zero to four participants (excluding body and clothing surfaces)

Three surfaces were not touched during the experiment, the cleaned vase and the kitchen cupboard handles which were not cleaned prior. No DNA was detected on the vase, 0.9 ng was found on the upper cupboard surface, and 1.8 ng was found on the lower cupboard surface. The male and female hosts were detected on the cupboard areas, which is expected from their presence in the home over time.

The majority of surfaces that were contacted by one participant (that coincidently were the also last contacts) resulted in detection of the contacting person (82 % or 36/44 of the samples). For the remaining samples where contact did not result in detection, one sample (13 %) was contacted by female host, none were from surfaces that were contacted by the male host, three (38 %) were contacted by the female visitor, and four (50 %) were contacted by the male visitor. The visitors favoured exclusion on all areas where they sat (table surface, back of chair and the chair seat; one or both hosts were detected in all of these samples) for the duration of the experiment, while the male host was included in two of three samples and the female host in two of three samples (one of which favoured inclusion for the male host), likely overwhelming any single use deposits of the visitors (discussed in previous section).

An interesting item to assess was each participant's glass that was new and purchased for the experiment. All participants made larger number of contacts with their glasses during the sitting, while no other individual contacted the glasses, and were all detected on these items, being either major or sole contributors except for the female host who was detected as a minor contributor. The accumulative duration of contact for the male host and visitor was 53 seconds and 26 minutes and recovered 2.8 ng and 9 ng of DNA respectively. The female host made 34 contacts, lasting cumulatively for 4 minutes 46 seconds, with her glass and contributed 0.09 ng of DNA. Notably, the male host, who did not make contact with the female host's glass, w as detected in this sample (LR1E06) (see Section 4.4 for further discussion). The female visitor (another low shedder) contacted her glass for 11 minutes 16 seconds, yet deposited 0.38 ng of DNA, lower than the quantities detected from the male higher shedders.

Overall, for surfaces contacted by one participant, another (non-contacting) participant was detected in 50 % of the samples. The other individual was predominantly one of the two homeowners.

Surfaces contacted by two participants (n = 11) resulted in detection of both in 18 % of the samples while the remaining samples detected one of the two contacting persons. The last person to contact the surface was detected in 91 % of the samples (10/11). Further, the female host (designated as a low shedder), was excluded (or favoured exclusion) in 90 % of samples contacted by her and one other person (10/11 samples in this category where she was excluded from 9/10 samples), but the duration of contact was less than a minute. A non-contacting person, the male host, was detected in four of these samples, with the longest duration and most contacted handler also detected, while the second handler (female host) was excluded or favoured exclusion in 75 % of these samples.

No surfaces were touched by three participants only and nine surfaces were touched by all four participants. None of the items in the latter category resulted in the detection of all four of the contacting people. The last contact was detected in 78 % of the samples (7/9). There were two items where three contacting people were detected, the jar (male host and two visitors) and the toilet door handle (hosts and low inclusionary support for the male visitor). Two of the contacting people were detected in 56 % of the samples and one contacting person was detected in the remaining 22 % of samples. For six of these items no contact history is available as they were taken from inside the toilet (Supplementary data 4; only hosts detected in these samples). The remaining three items were game dice, jar and jug, with jar and game dice being new and purchased specifically for the experiment. The dice was contacted for approximately the same time by all participants (<46 seconds), yet only the male host and female visitor were detected. When looking at the last five contacts with this item, the female visitor was the last person to make the contact (order: male visitor, female host x 2, male host and female visitor). Similarly, the jar and the jug were contacted for less than a minute by each individual participant (but greater than one minute for all individuals combined) and either both male participants (jar; male host last contact and last five contacts: female host, female visitor, male visitor, female host and male host) or male host only (jug; last five contacts identical to jar; however, this item is likely to contain host background DNA) were detected. Of note, the male host was detected on all nine samples in this contact category.

A small number of the samples were from the visitor's houses (n=8) and cars (n=6; Supplementary data 4). Neither the hosts nor the other participant were detected in these samples. The visitors were detected in all of their own samples with the exception of the male visitor home toilet button where a two- person mixture of unknown contributors was obtained. The roommates of the male visitor were detected in two samples (on the female visitor's car door handle and on the inner door handle of their house), however neither of these two individuals was detected in any of the samples taken from the experimental house. The female visitor's partner was detected in several female visitor home and car samples (see Supplementary data 4); as well as her clothing, intimate samples (see Section 3.5) and an experimental mug (see Section 4.3).

In general, results show that presence or absence of background DNA can be an important factor for the possible detection of the foreign, to the environment, DNA. Irrespective of the number of contacting people (and excluding own items such as home and car items), visitors were detected on surfaces that were less likely to contain high levels of background DNA (e.g. glass (1 contact; table 2 of Supplementary data 4) and fork (2 contacts)) and absent from surfaces that are rarely cleaned and thus likely to have higher levels of background DNA (such as chair seats and table (1 contact) and bathroom towel (4 contacts)).

Unknown contributors were detected in 34 % (43/127) of all samples and 41 % (27/66) of non-intimate samples (excluding clothing and body samples). The majority of these samples were items that were in general regular use and touched by multiple participants including chairs, handles, table etc. (Supplementary data 4). In this study, the unknown donors were observed as a single source or a majority contributors in six of the samples (5 % of profiles). These samples were collected from a number of items within the visitor's homes, including the toilet button, interior door handle and a number of car samples including steering wheel, car seat and door handle. Reference samples were obtained from the visitor's close associates however there were regular visitors to the premises from whom reference samples were not available. Also, these sample types are exposed to situations that are likely to accumulate greater levels of background DNA.

3.5. Clothing and intimate samples

3.5.1. Clothing items

On average, underwear (excluding gusset; due to differences in female/male biology) of the female and male visitors had 1.2 ng and 0.09 ng of DNA respectively and male and female hosts had 2.8 ng and 0.25 ng of DNA respectively. Of the 34 clothing items, the wearer was detected as the single, major or majority contributor in all but two samples (6 %) (outer back of pants of female host and male visitor's outer right sleeve) (Supplementary data 4). These samples were from outer clothing, such as sleeves, surfaces that are not in direct contact with the body and are exposed to the external environment. Male host was detected on two female host's clothing items (back of pants and underwear gusset). The male host was also present on the female host's chair (backrest and seat), suggesting indirect transfer to the back of her pants, being the major contributor to both of these samples. Conversely, female host was detected as a minor contributor to the back of male

host's pants, and his chair seat (but not to the backrest). Similarly, female visitor's partner (not present during the study) was detected on 88 % of her clothing including back of pants, and internal and external wings of the underwear. Unknown contributors were also present in 35 % of the clothing samples (pants, sleeves and underwear), including two samples as the sole contributor (male visitors right sleeve and underwear internal waistband).

3.5.2. Hand fingernail and intimate samples

Hand and fingernail samples of the two male participants produced single source profiles or mixtures with over 95 % contribution from the donor. The hand and fingernail samples from the two female participants (both low shedders) had more variable results. The female visitor was detected in all four samples (91–100 % contributions) while the female host was detected in 3/4 of her samples. Her left-hand produced a three-person mixture with the male host and unknown donor and her left fingernails sample was a partial single source unknown profile (9 alleles). Male host was detected in both hand samples of the female host. As part of the instructions, all participants washed hands (post toilet visit) in the middle of the experiment and video recording showed that she did not directly contact the male host after return from the bathroom break; suggesting that his DNA was indirectly transferred to her hands from one of the contacted surfaces. The last few contacts that this participant made, excluding touching herself, before sample collection, were the table and her glass both of which detected male host as the major contributor. However, it cannot be completely excluded that his DNA was present on the female host's hands from a previous direct contact and not completely removed during the handwashing.

For three of the participants, forearm skin samples were taken (surface in contact with the table). Similar to the hand samples, the donors were always detected as major contributors. Further, one of these samples produced mixtures with one unknown contributor likely picked up from the environment. The majority of hand samples also produced mixtures (62 %); however, of these, 60 % were attributed to live-in partners of the donors and likely picked up from environments and direct contacts. Conversely, 63 % of the fingernail samples were single source profiles matching the donor. Overall, unknown DNA was detected in 45 % of the fingernail and hand samples (Supplementary data 4), in all but one sample as a minor contributor ranging from 1 % to 16 % (av. 9 %).

Intimate samples from both males resulted in single source profiles matching the donor in all but one sample from the male host's shaft where the researcher was not excluded as a minor contributor (LR 7e05), possibly from contamination during sample processing (Supplementary data 4).

3.6. Male DNA detection in female samples

Of the 22 samples processed with Yfiler™ Plus (hands, fingernails and underpants from the female participants), four samples did not produce a profile, all of which were underpants samples (Supplementary data 4). The DNA amounts ranged from 0 ng to 2.5 ng (av. 0.43 ng; Supplementary data 4). Of the 18 samples that did generate profiles, 17 % were full single source profiles. The remaining samples were partial single source (50 % of samples) and two person mixtures (33 % of the samples). Two partial profiles had 1 allele each and multiple participants could not be excluded as the source of the DNA in these samples. Five mixtures (5/6) could not be resolved into the major and minor components. In casework, these samples are commonly reported as unsuitable for further interpretation, however, for research purposes, these samples were analysed to determine whether the persons of interest were included or excluded.

While the male visitor was excluded from all samples, the male host was not excluded from three samples, including the female host's hands and the gusset of the underwear (YHRD range 1 in 645–5276). However, male host was also detected in the same samples with autosomal STR analysis (LRs range 2E7 to 10E14).

The female visitor's partner was not excluded from 10 samples collected from the female visitor's hands and underpants (YHRD range 1 in 4 to not observed in the database of 58031 respectively), however he was also detected in 6 of these 10 samples with autosomal STR analysis (LR range 2E05 to 8E12). Of interest, this person was excluded from the female visitor's underpants gusset with autosomal profiling but gave a strong inclusionary result with Y-STR analysis. Two of the four samples where the female visitor's partner was not detected with PowerPlex21 had some of the highest amounts of DNA detected in this study (female visitor fingernails and underpants gusset, 36 ng and 45 ng respectively), likely swamping traces of male DNA during autosomal profiling [46,47]. Finding background levels of cohabitating male's DNA on female underwear has been reported previously [48]. The study found transfer of cohabiting male's DNA is common and direct and indirect transfer opportunities arise with normal contacts between partners in shared home environment. Finally, traces of unknown male DNA were also detected in nine of the samples (allele range 1–6).

4. Discussion

4.1. Influence of shedder status, background DNA and number and duration of contact

The results of this study indicate that DNA yields are likely to be influenced by both the presence of background DNA from previous contacts as well as the shedder status of the participants. The female host was determined to be a low shedder, possibly explaining the predominance of her samples in the low total DNA amounts category. However, the male visitor's high shedder assessment was contradicted by many low total DNA results associated with this participant. The majority of these samples were from the clothing that would have been in contact with parts of the body other than the hands. Excluding external clothing samples that collect environmental DNA (e.g. pants), and are not in contact with the body, and the gusset region of the underwear (due to differences in male vs. female biology), on average male visitor had the lowest amount of DNA detected on their clothing compared to the other three contributors. It is possible that shedding ability from hands and the rest of the body are different, and one cannot be used to make inferences for the other. It is not surprising to find different types of shedders in the middle quantification category of 0.3–3 ng of total DNA. It can be expected, in general, that high shedders will be mostly found in intermediate and high quantification categories and low shedders in low and intermediate ones. This is in line with findings from shedder studies [18,35,45] that show that individual deposits, for any particular shedder type, can be placed into a different shedder class due to intra-personal deposit variability. Further, the history of item use, such as recent washing, exposure to the environment, surface type as well as frequency, duration and manner of use, are likely to have further contributed to the variability of the results [31]. If the variable in question has a negative effect on DNA deposition, conceivably, low shedders may not be detected and high shedders may be detected in a lower quantification range. Further studies are desired to determine the associations between the shedder status and the quantities of DNA left on items after regular use. Conversely, the largest number of samples in the high quantification category were from the higher shedder male hosts. It should also be noted that for the two low shedder female participants, when body and clothing samples are excluded two of the five samples in this category are likely to be contaminated with traces of saliva (e.g. glass) and two sample have another person (partner) as the major contributor (steering wheel and door handle). There was only one sample with high DNA quantity associated with the female host designated as a low shedder individual (underpants gusset). This may indicate that the other items within her home are saturated with DNA from the co-habiting male host, designated as a higher shedder, who is likely to be the dominant contributor to DNA profiling results. This was

demonstrated in 92 % of samples taken from surfaces within the home (Supplementary data 4). This was also observed in all four environmental background DNA samples from within the home where male host contributed 63–94 % of DNA.

Looking at the number of contacts made, the female host, due to the assigned host duties, has made the largest number of contacts with the target surfaces. However, this participant was excluded from 44 % of the touched surfaces. In general, detection post contact, was similar for the two female participants and the male visitor (38–44 %). In contrast, male host was detected in all surfaces that he contacted. The difference in the detection between the two male participants, intermediate and high shedders, is likely from the contribution of the background DNA where male host is likely to be well represented. The shedder contributions can be seen in the quality of the profiles obtained when the participants were detected after direct contacts. The two low shedding females were minor contributors in 31–94 % of the samples while the two higher shedding males were minor contributors in 0–7 % of the samples. These results suggest that the shedder status and the presence of background DNA, as a consequence of multiple previous contacts, are likely key variables for the detection and the quality of the profiles generated. This is supported by detection of male host's DNA on 20 items, that they did not touch during the experiment, likely from background. Combination of background DNA along with subsequent contacts (during an experimental hour) from an intermediate shedder (male host) likely resulted in higher detection of this person on the tested items. Conversely, high shedder male visitor, who did not contribute to the background DNA, was detected less frequently.

Interestingly, the two visitors were excluded from their chair samples while making contact with these surfaces for most of the experiment. Hosts were detected in the chair samples suggesting that with larger surfaces, that have been in common use by an owner, short term use, even if close to an hour, may not be sufficient to replace background DNA present. Goray et al. [28] investigated the detection of the intruder's DNA to single occupation offices and found that larger surfaces were more likely to produce profiles inclusive of the usual owners or users while smaller items were more likely to detect last user's DNA, even if not the usual owner. Atkinson et al. [49] tested prevalence and persistence of DNA on regular use items after a short or one-time use by a second individual. Present study, concurring with previous research [49–52], found that habitual user's DNA was found on most items as a major contributor, regardless of the subsequent short contact by another person. This indicates that, in criminal investigation, when targeting a one-time user (for example in an assault case), in the first instance, items of relevance that are likely to have lower levels of background DNA should be targeted.

While both duration and number of contacts did not significantly affect the amounts of DNA detected, background DNA was a meaningful contribution factor. Such background is likely a result of previous, undocumented contacts by the participants with the sampled surfaces. Thus, it is likely that prolonged, multiple, previous contacts allow for greater detection of DNA. When evaluating possible detection of the person of interest on an item in question, both previous use or ownership of an item and the person's shedder status should be considered together. For example, an intermediate shedder in combination with a high level of background DNA can result in high level of detection (MH (detected on all items touched as sole or major contributor) than a high shedder in combination with a low level of background DNA (MV; detected in 56 % of touched items mostly as a sole or major contributor (93 %)). The data, such as the one presented here, can be mined to create datasets for activity level evaluations based on this knowledge and the case scenario at hand. In a hypothetical case involving a high shedder and an unknown (to the POI) environment would require the types of data that were obtained from the male visitor.

The last contact has not always resulted in detectable transfer (8–22 % of samples contacted 1–4 times). In many of these samples, the last contacting persons were female participants, again suggesting that shedder status may play a role. Likewise, several studies have investigated the DNA contributions to surfaces based on duration and sequence of contacts by different people and found that the last contact does not always result in the major contribution or detectable transfer [28, 52–55]. The non- contacting person was detected on 50 % of items touched by a single participant. While their presence may be due to secondary transfer events via intermediaries, the major reason is likely to be their presence on these surfaces and items from prior to experiment commencement (i.e., as background DNA). Detection and influence of background DNA has been discussed earlier in the discussion and reported previously [28]. There were three instances which indicated an indirect transfer event, based on the inclusionary support for the male host on the female host's glass and mug; and for the female visitor on the male visitor's glass. This accounts for 7 % of the samples contacted by just one person. For all items contacted by two people, indirect transfer was observed 4/12 times (33 %) which is higher than items only contacted by one person (7 %). All indirect transfer observations, when items were contacted by two people, related only to the male host, although this may indicate the presence of background DNA. Genuine indirect transfer could not be confirmed on any item contacted by two people. None of the items was contacted by three participants, and items touched by all four could not be assessed for the presence of the non-contacting person. Further indication of indirect transfer stems from the detection of unknown DNA in the majority of samples in this study, including clothing, underwear and hand and fingernail samples. Unknown DNA is commonly found on touched surfaces, usually as minor component to that of the usual user or wearer [33,34,53,56]. It should be noted that while the underwear was new and wrapped in plastic, it was not sterilised and hence the possibility of low-level background from manufacturing processes is possible. A recent study by Oefelein et al. [57] found that 70 % of samples taken from new, unwashed underwear that was handled exclusively by female participants contained trace amounts of male DNA. This observation could occur as a result of background DNA from events that occurred prior to any experimental study or due to indirect transfer from hands or contacts with surfaces such as clothing and toilet seat.

4.2. *Clothing, body and intimate samples*

Variability of the quantification results observed in the clothing samples may be due to a number of factors. The history of wear (post washing) for most of the items was unknown and this is recognized to influence the types of the results obtained [58–61]. Additionally, the shedder status of the participants (see Section 3.1.3) and the sampling technique used may have played a role also. Some studies suggest that swabbing is not as effective as tapelifting or cutting out at collecting DNA form the fabric [60,62], however it should be noted that some of the clothing samples in this study generated up to 44 ng DNA using double swabbing technique; stuffiest for standard DNA profiling. Not surprisingly clothing owners were detected on the majority of their own items. Frequency of detection of a wearer, a toucher or other and persistence of wearer on underwear was tested in the study by Breathnach et al. [27]. During the persistence part of their study the wearer was detected in 87 % of the samples, similar to this study where the wearer was detected in 94 % of the samples. Notably, both studies found the wearer was the major contributor (with the exception of the back of the female hosts pants). The male and female individuals were the sole contributors on their underpants in 64 % and 14 % of samples respectively. Of note, Breathnach et al. [27] found that 11 % of the study samples resulted in the reportable profiles of the toucher (after a 15 second contact). While in present study the underwear or clothing was not directly touched by any individual other than the wearer (i.e. no 'toucher') and participants were directed not to engage in any direct physical contact with other individuals, similar results were observed, with DNA from another participant or participant associate observed in 24 % of clothing samples (3/8 of which were external clothing surfaces

and 5/8 were from underpants). Szkuta et al. [50,58] studied transfer and recovery of DNA on the upper garments; to assess how daily activities influence the presentation of the background DNA on outer surfaces. The study showed that the wearer is often found as a single or major contributor, however, these proportions differed depending on the external environment and exposure and wearer's associates were also detected in a number of samples. Associates, such as children, partners and colleague's or unknown DNA can be picked up on outer clothing from common surfaces such as furniture [58,59]. Further, families usually wash their clothing together and this could have resulted in transfer between clothing items. Multiple studies have found that sufficient amounts of DNA, to generate a DNA profile, can be transferred between freshly laundered items [63,64]. Further, a study by Jones et al. [65] investigated transfer of female DNA to male intimate areas and underwear through non-intimate physical social contacts. In one part of their experiment, males simulated urination immediately after the "non-intimate" contact, but the penile and underwear samples were collected 6 hours after, similar to the present study. Comparable to the present study, researchers found female participant on one male underwear (7 %) and none of the penile samples. It should be noted that contrary to this study, Jones et al. [65] asked male participants to rub hands and faces of their female counterparts, while this study requested participants to refrain from any direct contacts with each other.

Similarly, donors of body samples were detected in the majority of their own samples, mostly as sole or major contributors. Detection of foreign (known and unknown) DNA was lower for fingernail than body samples. In agreement with present research, studies on the prevalence of foreign DNA under the fingernails in general and co-habituating populations found non-donor DNA in 5 – 41 % of the samples and showed that complex mixtures (greater than two people) and mixture inversions were rare [31,66–72]. The results of the clothing, hands/-fingernails and intimate samples show that, not surprisingly, the donor or the wearer is usually detected as the only or the main contributor to these samples. The detection of the known, to the donor, person(s) in these samples was also expected and noted. The rate of their detection can be incorporated into the activity evaluation probability tables when accounting for social interaction transfer probabilities.

4.3. Activity level evaluations

Here we provide one example of how our data (Supplementary data 4) may be used during these types of evaluations. An example scenario is an assault alleged to have occurred at a social gathering. Consider the scenario where the defendant attends a social evening at the home of the complainant. During the evening the complainant and defendant (along with other individuals at the gathering) play games, eat, drink and use the bathroom. The complainant states that at the end of the evening they got into an argument with the defendant, who ended up threatening them with a knife taken from the kitchen table before running away. The complainant immediately reported this to the police who attended the scene and seized the knife. The police arrested the defendant, who denies threatening the complainant with the knife.

The propositions for this case are:

H1) The defendant held the knife during the time the complainant was threatened

H2) The complainant was not threatened with a knife by anyone during the social interaction

Background:

- The defendant was at a social gathering at the complainant's home for approximately 1 hour, during which he ate food, played board games, drank and used the bathroom

Assumptions:

- There was no recent contact between the defendant and complainant prior to the social gathering

When the knife was examined at the forensic science facility, a sample from the handle yielded a complete, single-source DNA profile that matched the reference DNA profile from the defendant. We will only consider the presence or absence of DNA in this evaluation and so DNA amounts (and mixture proportions if we were dealing with a mixed DNA profile) do not need to be considered. There is no dispute over the source of DNA, and it is assumed that DNA from the defendant is present on the knife handle.

We define the following terms:

s - the probability that DNA from the defendant transferred to the knife handle during social interaction

p – the probability that DNA from the defendant transferred to the knife handle during it being used by them to threaten someone

There are three ways in which the defendant's DNA could have come to be present on the knife handle under the prosecution proposition:

- The DNA transferred during social interaction, but not during the alleged threatening, $s(1-p)$
- The DNA transferred during the alleged threatening, but not during the social interaction, $(1-s)p$
- The DNA transferred during both social interaction and the alleged threatening, sp

Under the defence proposition, DNA has transferred during social interaction (and the alleged threatening did not occur) leading to probability, s. This leads to:

$$LR = \frac{s(1-p)+(1-s)p+sp}{s} = \frac{s-sp+p}{s}$$

Therefore, we need to use data to inform two probabilities, s and p.

We will consider two different variations of this case, the first is when the defendant claims that they did not touch the knife, allegedly used to threaten the complainant, and the second is when the defendant claims that the knife used to allegedly threaten the complainant was the knife he used during dinner.

Considering first that the knife used in the alleged assault was not used by the defendant. From the experiments performed in our study we can obtain s by considering the DNA samples taken from the cutlery of all individuals, and noting in how many of these had DNA from individuals that did not touch them during the evening. From the four forks used in the experiment there are 12 opportunities for people's DNA to be indirectly transferred (i.e. FH, MH and FV onto the fork of MV; FH, MH and MV on to the fork of FV, etc).

Out of these 12 opportunities, indirect DNA transfer was observed once (MH's DNA on FV's fork). If we consider that there are two categories of outcome (DNA transferred, and DNA not transferred) then by adding a prior count of 1 to each category yields posterior probabilities of $s_i = (1+1)/(12+2) \approx 0.14$ (we use the subscript 'i' here to denote an indirect transfer, which will become important as the example expands).

To obtain the probability for p, we use the work of Samie et al. [73] who found DNA of individuals who had simulated stabbing were observed in 61 out of 64 samples from the knife handle. Adding a prior count of 1 to each category yields posterior probabilities of $p = (61+1)/(64+2) \approx 0.94$.

Using these values in the LR equation above would yield LR ≈ 7. In other words, given that the defendant was socially interacting with the complainant in her home, the probability of detecting the defendant's DNA in the knife handle is approximately seven times higher if the defendant held the knife during the threatening, rather than if he did not.

Consider now the same alleged scenario but instead that the knife used in the alleged threatening was the knife used by the defendant during dinner. This now requires a different value for s, as it must encompass the probability of DNA transfer during use. From the fork samples, the user of the forks yielded inclusionary LRs in four out of four samples. This leads to $s_d = (4+1)/(4+2) \approx 0.83$ and LR ≈ 1 (this time the subscript on 's' is 'd' to denote a direct transfer). As expected, the

higher probability of DNA from the defendant being present from social use of the knife means that the presence of his DNA on the knife handle has less power to discriminate between propositions.

4.4. Detection of indirect transfer

Most of the surfaces in this study had background DNA and thus it is conceivable that this background DNA was picked up and indirectly transferred to the sampled surfaces. While all of these instances cannot be determined with the use of video recordings, on a number of occasions, clear lack of direct contacts of the participants with the surfaces where they were detected indicated that indirect transfer was a mode of deposition. Indirect transfer was observed in 7 % of the samples (9 samples). This indirectly transferred DNA was a minor component in 88 % of the samples (3–30 % Mx contribution).

Female visitor's partner, who was not part of the experiment, indirectly transferred to five of her items including four underwear samples and the mug that was touched for the first time eight minutes into the experiment (48th contact made). The partner was also detected on the female visitor's pants, possibly as background, and consequently may have transferred to the external parts of her underwear. However, indirect transfer of the partner was not observed on the female visitor's chair. Of note, female visitor's partner was also detected in 5/6 body samples including hands and fingernails that may have acted as vectors of transfer.

The remaining indirect transfer samples were associated with the male visitor and the female host. A relative of one of the hosts was detected as a minor contributor on the back of the pants of the female visitor. This relative was detected as a major contributor to the sample collected from the chair suggesting indirect transfer with chair as an intermediate. The male host was detected on the gusset of the female host's underwear as well as her glass. Male host was detected as the majority contributor (66 %) to the glass sample. Yet, female host was a minor component with low inclusion support (LR = 10) on her own glass. The underwear and the glass were new and not directly contacted by this participant. However, male host was also detected on the female host's hands (sampled at end of experiment) that likely acted as vectors of transfer. The last item that the female host touched prior to hands being sampled was her skirt and male host was detected on this item. One explanation for this finding is that the female host, being a low shedder, acted as a vector of indirect transfer of the higher shedding male host. Goray et al. [35] investigated proportions of self and non-self DNA in hand deposits, finding that poor shedders may on occasion (approx. 3 % of all samples in that study) deposit only non-self DNA. Video recording showed that prior to touching the glass for the last time, female host touched the table section where male host was detected as the major contributor; possibly picking up his DNA and transferring it to the glass surface.

The female visitor was detected on the male visitor's glass; however, she did not have any direct contacts with this item. Review of the recordings showed that there were multiple instances where the male visitor contacted items and surfaces immediately after the female visitor including the jug, the jar and the bathroom towel and contacted the glass a short time later (2nd contact after touching the jar and the jug). During these occasions he may have picked up her DNA and transferred it to his glass. However, female visitor was not detected on the male visitor's hands, possibly lost during contacts subsequent to contacting the glass (two contacts with his phone), or the relative number of cells was masked by the classified shedder status of the male (high vs low).

5. Conclusions

This study provides a set of DNA data that can be obtained from surfaces contacted during a simple social interaction when all contacts are documented and assessed against the DNA profiles generated. Direct contacts did not always result in the detectable transfer and neither duration nor number of contacts had a significant effect. This data indicates that, when assessing the likelihood of detecting person of interest's DNA after a particular "crime associated" action, possibility of background contribution should be taken into account. Further, when determining the probability of transfer, shedder status of participants in combination with the presence/absence of background DNA were relevant factors.

Several instances of indirect transfer were observed, highlighting that indirect transfer is a common phenomenon that needs to be considered in criminal investigations. Importantly, transferred DNA was detected as a minor component in most samples. Further, detectable levels of participant's DNA did not move to a new location, beyond the experimental house; as evident from samples taken from the visitor's homes. Yet, visitors introduced their partner's DNA to the social interaction location.

Limited studies have utilised the aid of video recordings to better understand the movements of DNA [56]. This research further demonstrates that video recordings can introduce a new dimension to DNA-TPPR investigations allowing for better understanding of the DNA movements. Further studies replicating the conditions tested here and extending to different social circumstances will further expand the growing body of knowledge in this area. In the future, video recorded data analysis may be augmented with the use of AI and machine learning that may be able to pick up on the level of detail that the human brain cannot.

CRediT authorship contribution statement

Amy Cahill: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Luke Volgin:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Formal analysis, Data curation, Conceptualization. **Mariya Goray:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Formal analysis, Data curation, Conceptualization. **Roland A.H. van Oorschot:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Formal analysis, Data curation, Conceptualization. **Duncan Taylor:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation.

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.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.fsigen.2024.103101.

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