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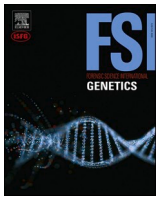

Transfer and persistence of intruder DNA within an office after reuse by owner

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ABSTRACT

The heightened sensitivity of DNA typing techniques, paired with the extensive use of trace DNA in forensic investigations, has resulted in an increased need to understand how and when DNA is deposited on surfaces of interest. This study focussed on the transfer, persistence, and prevalence of trace DNA in a single occupation of an office space by an intruder, when all contacts made during occupation and for the two hours prior and post occupation were known. The extent to which DNA could be recovered from contacted/not contacted surfaces was investigated. This study investigates the impacts of these movements and use of an office space when the duration of occupancy, surface contact histories and shedder status of participants are known. Contacts were documented and surfaces in the office space were targeted for sampling. Categories were set for target sampling that included different types of contact. Direct and indirect DNA transfer was detected in 55 % and 6 % of samples, respectively. Contactless DNA transfer was detected in 0.5 % of samples. The owner was observed as the sole/major/majority contributor in 77 % of the samples and as minor contributor in 10 % of samples. The intruder was observed as the sole/major/majority contributor in 14 % of samples and as the minor contributor in 16 %. An increased number of contacts increased the relative DNA contribution of the individual making the contact, however, not all observed direct contacts resulted in detectable DNA transfer. The outcome of this study will aid in better sample targeting strategies and contribute to the pool of data assisting in the development of activity level assessments.

1. Introduction

With improved sensitivity of DNA typing, the nature of samples collected from crime scenes has broadened from visible stains such as blood or semen to invisible sources such as trace DNA [1–3]. Currently, trace DNA samples taken from objects assumed to have been contacted by the hands or skin, and referred to as touch DNA, represent a large proportion of samples processed by forensic laboratories [2–5]. For example, in 2022, 62 % of evidence samples analysed at Forensic Science South Australia were touch DNA [6].

There are a multitude of variables associated with, and impacting, DNA transfer, persistence, prevalence, and recovery (DNA-TPPR). Understanding the influence of these variables is important, as different transfer scenarios may explain the presence of a person ‘s DNA on an item or surface of interest [2,7–11]. There are many pathways by which DNA can end up at a sampling location. Apart from direct deposition by a person of interest, their DNA could be present at a location that they had not been at, or contacted previously, through indirect transfer events [2].

To better understand DNA-TPPR, and the associated variables, a substantial component of the research has been conducted under controlled and semi-controlled conditions [7,12–17]. However, there is a recognized need to conduct more investigations in uncontrolled circumstances to mimic real-life scenarios [2,3,18,19].

A common scenario, relevant to casework, involves temporary use of an area known to be owned or predominately occupied by a single person (or a few people) such as an office space, or a house or residence, by an intruder. In such scenarios, an intruder may contact items and surfaces belonging to, and used by, the owner. After returning, it may not be immediately obvious to the owner that intrusion took place.

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Continued use of the space, until realisation, not only delays commencement of forensic investigation, it also potentially diminishes and/or alters evidence of an intruder's presence and /or actions. The extent to which an intruder's DNA, deposited during their visit, is lost from the location it was deposited on, transferred to another item/surface, and/or replaced by the returning owner is largely unknown.

There are several studies that have investigated the persistence of an original user's DNA on an item after use by another individual [17, 20–24] and the persistence of DNA from multiple sequential handlers of an item [1,24–26,719], but only a few studies have investigated the prevalence of DNA of known users within environments such as houses, offices, or cars [27–30]. A previous study by Goray et al. [27] investigated the relative prevalence on various locations of DNA from a temporary intruder within an office environment primarily used by one other individual. Here we investigate the relative presence and locations of DNA within similar office settings after use by a temporary intruder and a period of further use by the original primary owner. In contrast to the previous study by Goray et al. [27], this new study incorporated the use of video recording of all activities by participants in the office spaces, thus better informing ground truth, through the analysis of the contacts made with the targeted surfaces in the last three hours prior to sampling. The video recordings showed which areas should be targeted for sampling, including those with various sequences of known contact histories and areas known not to have been contacted during the recording period. The extent to which participants took DNA away from the office was also assessed by sampling hands, clothing, and in some instances faces, of intruders and owners (after the return visit) upon leaving the office. Improved DNA-TPPR knowledge, and data on profile types generated, in relation to items and surfaces within different spaces and circumstances commonly encountered in forensic casework will assist forensic investigators develop sample targeting strategies and conduct activity level assessments [8,11,31–33].

2. Materials and methods

2.1. Experimental set-up

Use of an office space by the office owner and an intruder was recorded by two video cameras strategically placed in the office to capture all movements and contacts. This experiment was conducted a total of four times using four different office spaces with different combinations of office owners and intruders. The intruders have never visited the owners' offices prior to this experiment. The offices were not cleaned prior to experiment commencement to reflect the presence of background DNA in real life circumstances (see Supplementary data 4 for history of office occupation). The owner of the office space entered the office with instruction to contact specific items and surfaces (i.e., open the door, turn on light etc.; see Supplementary data 1 for full set of instructions) before proceeding with normal, unscripted office work for one hour. The owner then left the office under specific instruction (Supplementary data 1). After the owner left the office, a cleaned DNA-free packaged chocolate bar was introduced into the office space for the intruder to eat. The packaging of the chocolate bar was cleaned with 1 % hypochlorite followed by 70 % ethanol. The bar was placed in a drawer by the researcher wearing full-PPE. The researcher contacted the external door handle to enter the office, then the cabinet handle to open and close the cabinet, and finally the external door handle again to close the office door while wearing gloves. An intruder then entered the office under a separate set of instructions (Supplementary data 2), before they proceeded with unscripted office work for an hour. The intruder was not wearing gloves. After leaving the office under the same instructions as for the office owner, the intruder's clothing, face, and left and right hands (separately) were sampled. Another cleaned DNA-free packaged chocolate bar was then introduced into the office space, into the same cabinet and in the same manner as described earlier. The original owner then resumed occupation of the office under specific instructions (Supplementary data 1), before proceeding with unscripted office work for another hour. After this final hour was complete, the owner left the office under the same exit instructions and their clothing, face, and left and right hands (separately) were sampled. During these occupations there were no restrictions placed on the participants re-contacting items they had been instructed to contact during their period of occupancy. The full detailed set of instructions for the owner and occupant can be found in Supplementary data 1 and 2. After the second occupation by the owner, the two video cameras were removed from the office space, recordings reviewed, and relevant information about contact history such as contacting hand, contact duration, and number of contacts was documented. Specific items and surfaces were targeted, based on targeted contact history (Table 1) for sampling. While it was expected that no visitors would enter the offices during the experimental period, no specific instructions to this affect were conveyed. A visitor did enter on one occasion: in office 3 the office owner had a visitor for a meeting during their second occupation of the office space. The visitor was present for ~20 minutes at the beginning of the one-hour owner occupation. They opened and closed the door contacting the external and internal door handles when entering and leaving the office. During their presence, they sat in a chair for visitors and contacted a part of a desk surface. These were the only areas they contacted and were sampled at experiment completion. The profiles from the surfaces contacted by this visitor are discussed in Sections 3.11 and 4. This visitor's reference profile was compared to all DNA results generated from office 3; with details only provided when detected. However, this visitor was also a known regular visitor to the office prior to commencement of the experimental period. Questionnaire relating to basic participant demographics and activities relevant to the experiment were also collected (Supplementary data 3 and Table 2 of Supplementary data 4). All eight participants and three close associates of the participants, such as live-in partners, provided reference samples in the form of a buccal swab (see tab two of Supplementary data 4). The experimental results were compared to the DNA profile of the individual collecting the samples and was excluded from all. Further, the shedder status of each participant was determined using the methodologies described in Section 2.2.3.

All samples collected as part of this project were obtained after informed consent from the volunteers involved in the study and with approval of the Flinders University Human Research Ethics Committee in compliance with the National Statement on Ethical Conduct in Human Research of the National Health and Medical Research Council, the Australian Research Council and Universities Australia.

2.2. Sampling and processing

2.2.1. Sample selection

Samples were selected in two ways: preselected and targeted, such that samples with distinct types of contact history were collected and analysed (Table 1). Preselected sample areas were determined before the experimental stage of the study as they correlated with previous studies [7,27]. Goray et al. [27] investigated intruder occupation of an office, without subsequent re-occupation by the owner, and targeted office chairs, internal and external doorhandles, light switches, pens, notepads, computer power buttons, keyboards and the mouses by instructing participants to contact these surface at particular stages of the experiment. Instructions similar to Goray et al. [27] were given to the participants in this study, in order to generate data comparable to their dataset. Other preselected sample areas included personal samples from participants' faces (the lower half of the face, including the cheeks, chin and the area between the nose and upper lip as a single sample), hands (left and right as separate samples), and clothing (the seat of the outer lower garment, and the lower back of the upper garment), as well as the DNA-free wrappers of chocolate bars placed into each office space. Additional areas were targeted for sampling after reviewing video recordings to ensure areas of varying types of contact history were included in this study. Each sample collected was categorised based on

Table 1
Interaction mode category description and the total number of samples collected across all four office spaces.

Interaction Mode Category	Type of Contact History	No. Samples Office 1	No. Samples Office 2	No. Samples Office 3	No. Samples Office 4	Total No. of Samples
1	Touched by the owner during the first occupation	1	1	2	3	7
2	Touched by the owner during the second occupation	7	6	2	2	17
3	Touched by the owner during the first and second occupation	1	2	0	3	6
4	Touched by the intruder only	9	12	7	4	32
5	Touched by the owner followed by the intruder	3	5	0	0	8
6	Touched by the intruder followed by the owner	1	3	4	7	15
7[a]	Touched during all three occupations	11	16	14	8	49
8[a]	Not touched during all three occupations	8	8	4	5	25
9[a]	Owner and intruder hands, face, and clothing	8	9	10	10	37
	Total	49	62	43	42	196

[a] 82 out of 196 samples were pre-selected (36 in category 7, 9 in category 8 and 37 in category 9). The 'targeted' samples fell within the other categories.

Table 2
Amount of DNA collected from surfaces and item within each office (excluding personal samples) originating from the owner and intruder, and the shedder status of the owner and intruder of each office.

Office No. (#samples)	Shedder Status		Amount of DNA recovered (ng)					
	Owner	Intruder	Minimum		Maximum		Average	
			Owner	Intruder	Owner	Intruder	Owner	Intruder
Office 1 (49)	Intermediate	Low	0.04	0.06	11.7	1.4	1.4	0.5
Office 2 (62)	High	Low	0.07	0.04	41.7	1.6	10.8	0.5
Office 3 (43)	Low	Low	0.03	0.05	3.4	6.8	1.03	1.2
Office 4 (42)	Low	Low	0.04	0.07	7.4	0.3	4.3	0.1

its contact history as per Table 1. The data collected from the video recording included the sequence of contacts, the number and duration of each contact per person and sampled area for each office and is available in Supplementary data 4. All the experiments were conducted within one month (April-May; with the outside temperature ranging from 15 to 20^0 C and outside humidity ranging from 48 % to 88 % (see Table 3 in Supplementary data 4)).

2.2.2. *Sampling method and processing*

For all sampling conducted, the researcher wore full PPE to limit the possibility of contamination. Each item and surface were wet and dry swabbed using viscose swabs (Forensic Swab L, Sarstedt).

Personal samples from participants and samples from the external doorhandles were collected directly after the experiments. The remaining samples from inside the office were collected after reviewing the video footage for targeted sampling; ~24–36 hours after last occupant leaving. Office spaces were locked and unoccupied until sampling.

Small items like pens, light switches, and drawer handles were swabbed over the entire surface areas. Items with a larger surface area of contact, i.e., the office chair seat, back of owner's or intruder's shirt, and computer monitor, were swabbed using a 20 cm×20 cm stencil placed in an area known to have been contacted. The stencils were used to

Table 3
Number of different profile types observed within each office per history category (see Table 1).

	Profile types																			
	Owner					Intruder					Knowns associates					Unknowns[a]				
	S	Mj	Ma	Mi	Ex	S	Mj	Ma	Mi	Ex	S	Mj	Ma	Mi	Ex	S	Mj	Ma	Mi	Ex
Office 1	4	18	14	1	8	1	3	0	4	37	0	0	0	0	45	0	2	3	75	8
Office 2	7	46	2	6	1	1	4	2	13	42	0	0	0	0	62	0	0	0	60	13
Office 3	1	6	17	8	11	2	5	4	8	24	0	0	1	4[b]	38	0	2	3	67	5
Office 4	1	15	16	4	6	0	4	2	6	30	0	0	0	6[c]	36	0	0	4	63	3
Total	13	85	49	19	26	4	16	8	31	133	0	0	1	10	181	0	4	10	265	29

	No profile	Number of profiles in each Interaction Mode Category								
		1	2	3	4	5	6	7	8	9
Office 1	4	0	7	1	9	3	1	10	7	7
Office 2	0	1	6	2	12	5	3	16	8	9
Office 3	0	2	2	0	7	0	4	14	4	10
Office 4	0	3	2	3	4	0	7	8	5	10
Total	4	6	17	6	32	8	15	48	24	36

S = Sole contributor/ Mj = Major Contributor /Ma = Majority Contributor/ Mi = Minor Contributor/Ex = Excluded

[a] All unknowns were counted in instances where one sample contained multiple unknown contributors; thus, producing larger number of unknowns than samples collected).

[b] Two of the samples from office 3 detected the owner's partner as a contributor, the visitor was detected as a contributor in the remaining three.

[c] The owner's partner was detected as a contributor in these samples.

standardise sampling areas to assist yield comparisons within this and other studies. Other items, such as desk surfaces were swabbed in the area contacted with an approximate 5 cm margin to ensure the full areas observed to be contacted were sampled (Column E in Table 1 of Supplementary data 4).

DNA was extracted with DNA IQ™ system in a final volume of 60 µl (Promega®), quantified with Quantifiler™ Trio DNA quantification kit (Applied Biosystems™), and amplified for 30 cycles with the PowerPlex®21 multiplex kit (Promega®). Separation and typing of DNA fragments was achieved with 3500xL Genetic Analyser (Life Technologies) and GeneMapper™ IDx with detection threshold of 175RFU (Applied Biosystems™) software. All samples were submitted to the amplification step, independent of their detected DNA concentration.

2.2.3. Shedder testing

Shedder testing was performed on all participants (office owners, intruders and the visitor of office 3 mentioned previously) following a combination of protocols used by Hartog [34], Johannessen [35] and Kanokwongnuwut [36]. Participants washed their hands for 20 seconds without soap and dried their hands with paper towels. Participants were instructed to carry out normal office work at their own workstation for 30 minutes but refrain from eating, putting their hands in their mouth, wearing gloves, or washing hands again. Participants then placed left and right thumbs onto two separate glass slides for 15 seconds with moderate pressure. Diamond™ Nucleic Acid Dye (Promega®) was applied to the glass slides to stain the cells that were then visualised under a Dino-Lite Microscope (Microscopemaster). This process was repeated three times for each participant within one day with a time interval of 1 hour between collections; generating a total of six thumbprints per participant [35,36]. Thumb prints were photographed at 50X magnification with Dino-Capture software using a grid system of 0.25 cm^2 squares to capture the entire print. These images were processed with ImageJ software (National Institute of Health), used for cell counting, to determine the average number of cells deposited per 0.25 cm^2 [34]. The total number of cells was counted for each thumb print and averaged based on thumb size. This was done by dividing the total number of cells counted in all squares by total the number of squares. Shedder status of 'low', 'intermediate', or 'high' was assigned to each participant, dependant on the average number of cells deposited, as per shedder classification system developed by Kanokwongnuwut et al. [36]: low shedders – equal to or less than 322; intermediate shedders – between 323 and 733; high shedders – equal or greater than 734.

2.2.4. Data interpretation and statistical analysis

DNA profiles were deconvoluted using statistical software STRmix™ 2.9 (New Zealand Crown Research Institute, ESR & Forensic Science SA). There were three samples in Office 1 that produced six person mixtures (desk surface, cabinet surface and tissue box; Supplementary data 4). For the purpose of this study only, an owner of the office was assumed to be a contributor to these samples (after confirmation via manual analysis) in order to run these samples through STRmix software. The remaining samples were analysed without assuming contributions from the participants. Participants' reference profiles were compared to profiles obtained from experimental samples and the relevant output including percentage contribution via mixture proportions, and likelihood ratio for each contributor were recorded (Supplementary data 4). Profiles from all research team members with access to the building were compared to all the generated DNA profiles, and all were excluded. The reported Likelihood Ratios were for unrelated 99 % lower bound. Reference samples were not obtained from any office cleaners.

When a mixed DNA profile was detected, mixture proportions and/or the amounts of DNA contributed by each donor were determined via STRmix deconvolution output. Contributors were designated as major, majority, and minor: the major contributor was defined as a person who has contributed equal to or greater than 70 % of DNA in the sample. If none of the donors contributed over 70 % of the total DNA, then the majority contributor was assigned to the profile. The majority contributor was defined as a person that has contributed the largest proportion of DNA to the sample but less than 70 %. In a two-person mixture the majority contributor was the person who contributes between 51 % and 69 % of the total DNA. In a mixture from three or more contributors, the majority donor contribution was more varied due to different proportions that could be contributed by all donors. Minor contributors were defined as donors that did not contribute the major or majority of the DNA to the sample.

2.2.5. Result interpretation

The raw data collected from the DNA profiles and the video footage was imported into the statistical software platform SPSS 25 (IBM) for statistical analysis. Relationships were compared using both a one-way and two-way ANOVA between the amount of total DNA and the total number of contacts; the total number of alleles and the total number of contacts; the amount of total DNA and the total duration of contact; and the total number of alleles and total duration of contact. The total amount of DNA was also compared across all office spaces using a one-way ANOVA analysis. The number of contacts and amount of DNA per participant, and the duration of contact and amount of DNA per participant were analysed using linear regression. These statistical tests allowed for the observation of the possible relationships between these variables.

When calculating the number of alleles from a person of interest (POI) detected in the sample, a DNA profile was designated as complete when alleles were detected at every locus and loci with single allele observed reached a homozygous threshold of 2000 RFU. If a single source profile had less than 40 alleles (excluding sex specific locus) detected it was designated as partial. If no alleles were detected, such profiles were designated as no profile. A mixed profile was considered partial if no alleles were detected at one or more loci. For the total number of alleles in the profile, without reference to POIs, each allele was counted once (without reference to homozygote threshold), after artefacts such as stutter, overstutter, double stutter were excluded from analysis.

The minimum number of contributors (MNC) to a DNA profile was determined manually. The locus (or loci) with the largest number of allelic peaks was used to determine the MNC, once all artefacts were removed from a profile, by dividing the total number of alleles at that locus (or loci) by two and assessing Peak Height Imbalance between the proposed peaks (with the PHI of $\geq$35 % for proposed allelic pair).

A POI was considered not excluded from a sample if a Likelihood Ratio equal to or above 1 from STRmix analysis was obtained. However, it is acknowledged that a low LR value could be seen as adventitious. All LR below 100 are highlighted in text, to bring attention of the reader to their possible adventitious nature, in the results section. All LRs calculated are found in Table 1 of Supplementary data 4.

When the POI was not excluded as a contributor, the number of POI alleles detected in the profile was counted. For homozygote loci, the allele was counted twice when the height of the allele peak exceeded the homozygote threshold of 2000 RFU. In the case of mixed profiles, the shared allele RFU values were divided by their respective mixture proportions, based on STRmix analysis output, to determine the RFU values for each individual contributor. In such instances, if the homozygous allele RFU remained above the homozygote threshold it was counted twice (Supplementary data 4). It should be noted that for mixed profiles with one known and one or multiple unknown contributors it was not known if one or more alleles were shared between these contributors. In such instances, STRmix mixture proportions were used to allocated RFU contributions to known and unknown contributors.

Average RFU contributions, across profile, from known contributors were evaluated by adding RFU values from the detected alleles and dividing by the number of alleles observed. In cases with multiple known donors detected, RFU contributions were shared as described

above. Manual average RFU contributions were compared to the STRmix output of predicted Template RFU (Supplementary data 4).

2.2.6. Mixture to Mixture Comparison

Mixture to mixture comparisons were performed separately for each of the four offices using STRmix software (point estimate without theta) with an LR cut off of 100,000. This was done to assist in the possible identification of common unknown donors in each office space.

3. Results

3.1. Office DNA quantities and shedder status of occupants

The total quantities of DNA, contributed by the owner and intruder, when retrieved from surfaces in each of the four offices, varied (Table 2). Prior to experiment commencement there were several target categories that we wished to sample based on the contacts made. Office 1, 3 and 4 participants made less contacts in some of the target categories resulting in less samples collected overall for these office spaces. There were differences in the amount of DNA detected in the samples taken from surfaces in the office spaces. However, no significant correlation between the DNA amounts and the duration of contact ($p=0.19$) (Supplementary data 5), the number of alleles and the duration of contact ($p=0.41$), or the number of alleles and the number of contacts was detected ($p=0.47$). Significant correlation was noted between the number of contacts and the amount of DNA detected as the number of contacts increased so did the amount of DNA detected ($p=0.001$). The RFU contributions by all participants also varied and were reflective of the amounts deposited by the donors (Supplementary data 4). Shedder testing showed that 6 of the participants were low shedders with one intermediate shedder and one high shedder. Shedder status of participants had an impact on the amount of DNA detected in samples collected in each office space. The office owned by a high shedder (office 2) had the highest average amount of DNA detected, while the lowest amount of owner derived DNA detected was from office 3 where both participants were determined to be low shedders. However, average amounts of DNA detected in office 1, where the owner was deemed an intermediate shedder, were similar to those of the low shedder office owners. Further, low shedder office 4 owner had higher average amounts of DNA detected than that of intermediate shedder. When assessing the influence that the participants' shedder status has on the amounts of DNA detected, significant relationship was only noted for office 2 ($p<0.001$). It is also noted that office 2 had several more samples collected compared to the other three office spaces. When reflecting on the unique samples collected from office 2, they consisted of a mix of item/surface types, sizes and histories similar to those collected from other offices, therefore the average amount of DNA is deemed not to be inflated and the connection between the shedder status of the participant and the average amount of DNA not overstated. It should however be noted that office 2 owner occupied this office for the longest period of time and used it most frequently, compared to the other office owner participants (Supplementary data 4, Table 3).

3.2. General observations

Samples representative of target categories were collected from each office and DNA profiles were generated from all but four samples (Table 3). These four samples were all from office 1 and were collected from a laptop power cord (cat 1), the owner's shirt (cat 9), a light switch (cat 4), and a bookshelf (cat 8). Table 3 summarises the occurrences of the owner, intruder, known associates and unknowns, and their relative contributions, within the profiles generated per office. Overall, in each office, the owner was present in more samples than the intruder and was more prominent within the profiles generated. The owner was observed as the major/majority contributor in 70 % of the samples, sole source donor in 7 % of the samples and as a minor contributor in 10 % of the samples. However, there were a few occasions within the sample sets collected in each office where the intruder was either the only (4x), or major contributor (16x) to the profile. These samples tended to be from category 9 (13x), category 4 (6x), and category 7 (1x) and related to items/surfaces such as the participant's personal samples (face, hands, and clothing) (13x), the chocolate bars (3x), an internal door handle (x1), a coffee cup (x1), a book (x1) and a notepad (1x). Overall, intruder was detected as major/majority contributor in 12 % of the samples, sole source donor in 2 % of the samples and as a minor contributor in 16 % of the samples. Reference samples were available from the partners of office owners 3 and 4, and the known (regular) visitor to office 3, yet known associates were only detected on 12 occasions: the owner's partner in office 3 on the back of the owner's shirt (cat 9) and the phone speaker (cat 4); the visitor to office 3 being detected on a whiteboard marker (cat 2) and a chair base and backrest (cat 8); and the owner's partner to office 4 on the owner's shirt and left hand, the intruder's pants, and right hand (cat 9), an office chair (cat 1), a phone handle (cat 6), and a computer mouse (cat 7). In general, observed direct contacts resulted in detectable transfer in 55 % of the samples. DNA originating from unknown individuals were observed in 163 profiles, usually as a minor contributor (92 % of profiles) but occasionally as a major contributor (2 %) or majority (6 %) contributor. Two of the four samples where an unknown donor was observed as a major contributor were from office 1, on the intruder's shirt (cat 9), and a desk drawer (cat 4); and two from office 3, on the phone receiver (cat 4), and a folder (cat 4). Subsections 3.3–3.11 further consider the results of the samples collected per category.

3.3. Mixture to mixture comparison results

Mixture to mixture comparisons of the samples taken from office 1 showed that the same unknown contributor (office1 (O1)-Unknown 1 (UK1)) was detected on desk drawer 2, a paper bag and the owner's left hand (LR ranging from 1.2E05 to LR9E05; av. 5E05). No common unknown contributors were detected in the mixture-to-mixture comparisons of the samples taken from office 2 at the designated threshold. In office three, an unknown contributor (O3-UK2) was detected on the chair lever, the monitor screen face, pen 2 and the owner's back of pants (LR ranging from 1.4E05 to 2.6E07; av. 9E06). A different unknown contributor (O3-UK3) was detected in office 3, on the notepad, chair back and whiteboard marker (LR ranging from 2.8E05 to 2.8E07; av. 9.5E06). Finally, in office 4, a common unknown contributor (O4-UK4) was detected in the samples taken from the desk drawer, the book, the sticky notes, and internal and external door handles (LR ranging from 2E05 to 3.8E09; av. 1.2E09). Also of note, two of these samples had this unknown as a majority contributor (sticky notes and the internal door handle; LR 1E09). A different unknown contributor in office 4 (O4-UK5) was identified in profiles obtained from several items/surfaces: desk surface 2, computer mouse, desk drawer 1, intruder's face, the letter, office chair lever, the book, the whiteboard marker, desk drawer 2, desk surface 3, monitor screen, owner's right hand, owner's back of pants, pen 3 and external door handle (LR ranging from 1E05 to 3.9E12; av. 1.5E11).

3.4. Category 1 – touched by the owner during the first occupation only

There were 7 surfaces touched by the owners during the first hour of the experiment and not touched by anyone during the subsequent two hours. Of these 7 samples, 1 sample from a laptop cord (a light touch with the thumb and forefinger for ~2 sec) did not generate a profile and was excluded from further analysis. The owner was not excluded as the major or majority contributor in 5 samples (Table 4; all LRs $> 2E10^{8}$, av. of $4E10^{25}$). An unknown contributor was detected as a majority contributor in one sample (55 %; whiteboard marker 1) and the owner was detected as a minor contributor (36 %; LR of $3E10^{8}$). The intruder was excluded from all these samples (Supplementary data 4).

Table 4
Breakdown of DNA results obtained from Category 1 (excluding sample with no profile).

No. of samples	Contributor			Av. Owner DNA (ng)	Av. No. of Owner contacts	Av. Duration of Owner contact (sec/min)
	Owner	Intruder	Associate			
2	Major	Excluded	Excluded	1.1	5	10 min
1	Major	Excluded	Minor	0.9	3	4 sec
2	Majority	Excluded	Excluded	0.76	2	47 sec
1	Minor	Excluded	Excluded	0.5	2	2 min

A live-in partner of the office owner was not excluded from one sample (the front of an office chair seat). The results in which the close associate was not excluded had an LR of 3, which could be considered adventitious.

3.5. Category 2 – touched by the owner during the second occupation only

There were 17 items and/or surfaces only touched by the owners during the second occupation, i.e., not contacted by the intruder (Table 5, Supplementary data 4). The owners were not excluded as the sole, major, or majority contributor in all 17 samples (all LRs $> 9E10^{25}$, av. of $10E10^{25}$). The intruder was excluded from all samples in this category (Supplementary data 4). The known visitor to office 3, during the second occupancy of the owner, was detected as a minor contributor (LR of $1E10^{5}$) in 1 sample, from a whiteboard marker, contributing approx. 0.2 ng of DNA and 8 alleles. The visitor had not contacted the whiteboard marker during their visit. It is not known if they had touched the marker on previous visits to this office. Alternatively, secondary transfer could explain the detection of the visitor's DNA on this item, as the owner was observed to touch the desk surface that the visitor had touched after which the owner contacted the whiteboard marker. The owner was detected as the majority contributor in the sample from the whiteboard marker having used it once for ~19 seconds.

3.6. Category 3 – touched by the owner during the first and second occupation

Only 6 items/surfaces were touched by the owner only during the first and third hour of the experiment (Table 6, Supplementary data 4). The owners were not excluded as the sole, major, or majority contributor in all 6 samples (all LRs > 200, av. of $6E10^{25}$). The intruders were excluded from all samples in this category (Supplementary data 4).

3.7. Category 4 – touched by the intruder only

Thirty-two surfaces were only touched by the intruders during the experiments (Table 7, Supplementary data 4). The owner was not excluded as the sole, major or majority contributor in 23 samples (72 % of samples in this category) (LRs $> 3E10^{7}$, av. of $9E10^{25}$), as the minor contributor in 4 samples (all LRs $> 3E10^{7}$, av. of $2E10^{9}$) and excluded as a contributor in 5 samples. One sample in which the owner was not excluded as the sole contributor had an LR of 5 and could be considered adventitious. The 5 samples in which the owner was excluded were from two chocolate bar wrappers, a coffee cup, a phone receiver, and the inside of a notepad. Of these 5 items, it is likely that the owner had contacted two items, the coffee cup, and the phone receiver, prior to the experiment, however, the owner's DNA was not detected. The remaining three items were not expected to have been touched by the owner as the notepad page had not been written on prior, and the chocolate bars had been introduced to the office space, DNA-free, after the first hour of occupation.

The intruder was not excluded as the sole contributor in one sample (LR of 6; and therefore, could be considered adventitious). The intruder was also not excluded as the major contributor in 5 samples (all LRs $> 6E10^{22}$, av. of 1×10^{25}), and the minor contributor in another 7 samples (all LRs > 16, av. of $1E10^{11}$). Two samples in which the intruder was not excluded as the minor contributor had LRs of 16 and 20 and could be considered adventitious. The intruder was excluded as a contributor from 19 samples in this category (i.e., intruder contacted and was not detected in 59 % of samples in this category). When comparing the samples where the intruder's DNA was detected, most were obtained from items with small surface areas that were touched frequently for a duration longer than three seconds. This frequency of contact and small surface area may have allowed for the removal and replacement of the owner's DNA by the intruder. The surfaces contacted by the intruder, in which they were not detected, appeared to vary in surface area, frequency of contact, and total/individual duration of contact. One point of note is that surfaces where the intruder was not detected came from items that are likely to be consistently touched during a normal day of office work (i.e., phones, pens, desk surfaces, and desk drawer handles). Therefore, it is possible that areas contacted by the intruder may have been a relatively small proportion of the area possibly contacted by the owner prior to the experiment. A large proportion of these items were sampled in their entirety, picking up DNA from intruder contacted areas along with DNA present from previous owner contacts. In this category, it was observed that as the intruder's average number of contacts increased, the average total DNA contributed by the intruder increased as well. Excluding two samples that likely had saliva contributions (cup and phone speaker), average amount of DNA detected from the items contacted less than 4 times was 0.2 ng (n=7) while the average amount of DNA detected on items contacted 4–7 times was 0.5 ng (n=4) (Supplementary data 4). The level of contribution (major, majority, minor), also increased as the average number of contacts increased. Interestingly, one sample where both intruder and owner were excluded was the phone receiver, that was contacted by intruder for approximately 5 minutes, yet generated a mixture of two unknown donors. It is possible that it was used by one or both of these unknown donors for a prolonged period of time, but the use of the surface prior to experiment could not be ascertained for this item in our study.

Table 5
Breakdown of DNA results obtained from Category 2.

No. of samples	Contributor			Av. Owner DNA (ng)	Av. No. of Owner contacts	Av. Duration of Owner contacts (sec)
	Owner	Intruder	Associates			
3	Sole	Excluded	Excluded	1.4	2	7 sec
7	Major	Excluded	Excluded	2.3	2	11 sec
6	Majority	Excluded	Excluded	0.4	2	27 sec
1	Majority	Excluded	Minor	1.3	1	19 sec

Table 6
Breakdown of DNA results obtained from Category 3.

No. of samples	Contributor			Av. Owner DNA (ng)	Av. No. of Owner contacts	Av. Duration of Owner contact (min)
	Owner	Intruder	Associates			
1	Sole	Excluded	Excluded	0.06	123	53 min
2	Major	Excluded	Excluded	7.8	23	10 min
3	Majority	Excluded	Excluded	0.3	3	7 min

Table 7
Breakdown of DNA results obtained from Category 4.

No. of samples	Contributor			Av. DNA (ng)			Av. No. of contacts (Intruder)	Av. Duration of contact (Intruder) (sec/min)
	Owner	Intruder	Associates	Owner	Intruder	Associates		
2	Sole	Excluded	Excluded	11.9	0	0	2	2 min
8	Major	Excluded	Excluded	4.5	0	0	2	1 min
5	Major	Minor	Excluded	3.4	0.1	0	2	1 min
6	Majority	Excluded	Excluded	0.6	0	0	2	15 sec
1	Majority	Minor	Excluded	0.8	0.6	0	2	4 min
1	Majority	Minor	Minor	4	1.7	0.1	2	5 min
2	Minor	Major	Excluded	0.08	0.6	0	4	2 min
2	Minor	Excluded	Excluded	0.1	0	0	1	2 min
1	Excluded	Sole	Excluded	0	0.06	0	1	2 min
3	Excluded	Major	Excluded	0	2.6	0	5	30 sec
1	Excluded	Excluded	Excluded	0	0	0	2	5 min

3.8. Category 5 – touched by the owner followed by the intruder

There were 8 surfaces touched by the owner, during their first occupation followed by the intruder. These surfaces were not touched again during the final owner's re-occupation (Table 8, Supplementary data 4). The owners were not excluded as the sole and major contributor in all samples (all LRs $>$ 180, av. of $1E10^{26}$). The intruders were excluded as contributors from all samples in this category. Similar to Category 4, the surfaces sampled in this category are likely to be contacted frequently by the owner of the office space during a normal office workday. Therefore, it is possible that owner's DNA, from multiple previous contacts, overwhelmed lower amounts that may have been transferred by the intruder.

3.9. Category 6 – touched by the intruder followed by the owner

There were 15 items/surfaces touched by the intruders followed by the owners during their second occupation of the office (Table 9, Supplementary data 4). The owner was not excluded as the sole, major, and majority contributor in 12 samples (all LRs $>$ $3E10^{6}$, av. of $9E10^{23}$), as the minor contributor in 1 sample (LR of $9E10^{16}$) and excluded from 2 samples. One of the 2 samples (light switch) that the owner was excluded from corresponded with the detection of the intruder as the majority contributor and an unknown contributor; the other sample (computer power button) had intruder not excluded as a minor contributor and had one unknown contributor. Both items had a small contact and target area and were infrequently touched.

The intruder was not excluded as the majority contributor in 2 samples (all LRs $>$ $1E10^{14}$, av. of $2E10^{14}$), and the minor contributor in 5 samples (all LRs $>$ 2, av. of $6E10^{13}$). The intruders were excluded from 8 of the samples. Three of the five samples in which the intruder was not excluded as the minor contributor had LRs between 2 and 13 and could be considered adventitious. The surfaces where the intruder was not excluded were items contacted more frequently and for a longer duration during the intruder's occupation. These items were also observed to have a smaller surface area than the items where the intruder was not detected. Smaller surfaces may have made it easier to remove and replace the owner's DNA during contact. The intruder was not excluded from items that would not be regularly contacted by the owner, such as a book on a shelf and the outer edge of a monitor which was contacted by the intruder when looking for the power button. A live-in partner of the owner of the office 4 was not excluded as a minor contributor in one sample with an LR of 17 and could be considered adventitious.

3.10. Category 7 – touched during all three occupations

There were 49 items/surfaces touched by both the owner and the intruder during their 3-hour presence in the office. Of these 49 samples, 1 from a light switch did not generate a DNA profile and was excluded from further analysis (Table 10, Supplementary data 4). The owners were not excluded as the major or majority contributors in 41 samples (84 % of samples in this category) (all LRs $>$ 2, av. of $8E10^{25}$) and the minor contributor in 7 samples (all LRs $>$ 33, av. of $1E10^{15}$). Of note, the owner was not excluded in 4 of the samples with the LR ranging from 2 to 61 and can be considered as adventitious matches.

The intruder was not excluded as the major or majority contributor in 4 samples (all LRs $>$$1E10^{4}$, av. of $1E10^{23}$) and as the minor contributor in 14 samples (all LRs $>$ 3, av. of $1E10^{14}$). The intruder was excluded as a contributor in 31 samples. Of the 14 samples in which the intruder was not excluded as a minor contributor, 7 had LRs below 66, which could be considered adventitious. The items where intruder was not excluded were touched more frequently and for a longer duration by intruders when compared to the owners. This is in keeping with the results in the previous sections. These surfaces also had a small surface

Table 8
Breakdown of DNA results obtained from Category 5.

No. of samples	Contributor			Av. DNA (ng)		Av. No. of contacts		Av. Duration of contact (sec/min)	
	Owner	Intruder	Associates	Owner	Intruder	Owner (Pre-I)[a]	Intruder	Owner (Pre-I)[a]	Intruder
1	Sole	Excluded	Excluded	3.2	0	1	1	11 min	19 sec
7	Major	Excluded	Excluded	4.8	0	4	4	4 min	3 min

[a] Pre-I = pre-Intruder

Table 9
Breakdown of DNA results obtained from Category 6.

No. of samples	Contributor			Av. DNA (ng)		Av. No. of contacts		Av. Duration of contact (sec/min)	
	Owner	Intruder	Associate	Owner	Intruder	Intruder	Owner (Post-I)[a]	Intruder	Owner (Post-I)[a]
1	Sole	Excluded	Excluded	83.7	0	1	1	2 min	19 sec
1	Major	Minor	Excluded	4.0	0.1	2	3	3 sec	7 sec
1	Major	Minor	Minor	2.5	0.2	1	2	2 min	19 sec
4	Major	Excluded	Excluded	2.4	0	3	1	5 min	14 sec
3	Majority	Excluded	Excluded	0.2	0	4	1	2 min	5 sec
2	Majority	Minor	Excluded	0.5	0.3	1	5	3 sec	3 min
1	Minor	Majority	Excluded	1	1.6	2	2	4 sec	3 sec
1	Excluded	Majority	Excluded	0	0.07	2	1	4 sec	3 sec
1	Excluded	Minor	Excluded	0	0	19	2	5 sec	6 sec

[a] Post-I= Post Intruder

Table 10
Breakdown of DNA results obtained from Category 7.

No. of samples	Contributor			Av. DNA (ng)		Av. No. of contacts			Av. Duration of contact (sec/min)		
	Owner	Intruder	Associate	Owner	Intruder	Owner (pre-I)	Intruder	Owner (post-I)[a]	Owner (pre-I)[a]	Intruder	Owner (post-I)
21	Major	Excluded	Excluded	17.8	0	23	10	18	26 min	16 min	21 min
8	Major	Minor	Excluded	4.9	0.3	12	9	10	22 min	7 min	17 min
1	Major	Excluded	Minor	2.2	0	24	4	63	27 min	26 sec	35 min
6	Majority	Excluded	Excluded	0.2	0	3	3	2	1 min	1 min	1 min
5	Majority	Minor	Excluded	1.2	0.5	14	22	11	40 min	7 min	43 min
2	Minor	Excluded	Excluded	0.2	0	3	4	4	30 min	26 min	29 min
3	Minor	Majority	Excluded	0.4	0.6	7	7	3	2 min	1 min	2 min
1	Minor	Major	Excluded	0.07	0.9	1	2	1	3 sec	5 sec	2 sec
1	Minor	Minor	Excluded	0	0.1	1	7	3	1 sec	3 min	3 sec

[a] Pre-I and Post-I= pre and post Intruder

area making it easier for the intruder to remove and replace the owner's DNA with their own. A live-in partner of the office owner was not excluded as a minor contributor in one sample. This sample had an LR of 6 and could be considered adventitious.

In this category, it was observed that, in general, but not always, as one participant's average number of contacts increased, the average total DNA contributed by that participant also increased. The level of contributor (major, majority, minor), also increased as the average number of contacts increased. It was seen that if the intruder contacted an item on average more than the owner, the intruder would be observed to have contributed more DNA to the sample and vice versa.

3.11. Category 8 – not touched during all three occupations

Twenty-five sampled items/surfaces were not touched by the owner or intruder during the three-hour occupation of the four office spaces. Of these, 1 sample from a bookshelf did not generate a profile and was excluded from further analysis (Table 11, Supplementary data 4). The owners were not excluded as the sole, major or majority contributor in 18 samples (72 % of samples in this category) (all LRs $> 5E10^6$, av. of $1E10^{26}$) and were excluded as a contributor from 6 samples. These 6 samples were from two chair surfaces in the areas in which a visitor would sit, a main office chair lever (from chair used by the owner), a screen of a monitor, a cabinet surface, and a notepad.

Table 11
Breakdown of DNA results obtained from Category 8.

No. of samples	Contributor			Av. DNA (ng)	
	Owner	Intruder	Associate	Owner	Intruder
2	Sole	Excluded	Excluded	4.1	0
10	Major	Excluded	Excluded	5.5	0
1	Majority	Minor	Excluded	0.7	0.04
5	Majority	Excluded	Excluded	1.4	0
5	Excluded	Excluded	Excluded	0	0
1	Excluded	Excluded	Majority	0	0

The intruder was detected in one sample (LR of 100) in office 2. This sample was taken from a cabinet drawer handle. The drawer was located in the back corner of the office space and was the third drawer from the top, closest to the ground compared to the other cabinet drawers sampled in the same office space. The intruder was not observed to touch the drawer itself but had occupied that corner of the room (~1 m away from the sample) whilst taking a book off the bookshelf and stood there whilst reading it. The intruders were excluded from the remaining 23 samples. The known visitor to office 3, during the owner's second occupation, was not excluded as the majority contributor in a 5-person mixed profile from a visitor's chair seat they sat for ~20 minutes. The known visitor contributed 1.05 ng or 45 % of the total DNA (LR of 400). The owner and intruder were not detected in this sample.

3.12. Category 9 – hands and clothing samples (considered in combination with the results from the last five items touched by hands)

Thirty-two samples were collected from the participants' hands and clothing. There were 16 samples taken from the owners (after occupying the office for 1 hr immediately after it had been occupied by the intruder for 1 hr), and 16 samples taken from the intruders (after occupying the office for 1 hr immediately after it had been occupied by the owner for the first 1 hr).

The profiles generated from the last five items touched by the hands of the owners or intruders, just prior to leaving the office, were taken into consideration (Table 4 of Supplementary data 4) when assessing profile generated from hands. For intruder in office 2, where the owner was detected in both hand samples, of the last five items contacted most had the owner as the major or single source contributor (9/10 samples including surfaces such as keyboard, mouse, desk surface etc; see Table 4 of Supplementary data 4). For the intruder in office 3, where the owner was detected on the right hand, of the last five items contacted, the owner was detected in 4 of the 5 surfaces and as the major contributor in 2 samples, including the last surface contacted before sampling. These last contacts before sampling likely allowed for owner's DNA to collect

on the hands of intruders just before sampling took place. Not surprisingly, of the last five items touched by the owners, most had owners' DNA that was often represented as the major contributor. However, several items had intruders' DNA on them, mostly as a minor contributor. This likely resulted in accumulation of the owner's DNA on their own hands and downed out any traces of intruder's DNA that may have been present or collected from the last five items. The only instance of intruder detection on the hand of an owner of office 2 is discussed in the next section.

3.12.1. Owner samples

Owners were not excluded as sole or major contributor in all 8 samples taken from their hands (all LRs > $7E10^{13}$, av. of $1E10^{29}$). The intruder in office 2 was not excluded as a minor contributor in 1 of the 8 samples (office 2 owner's left hand) (LR of 600). The office 2 owner is left hand dominant and had contacted the external doorhandle directly before their hands were sampled. The intruder was also observed to touch this same doorhandle as the last contact when leaving the office space, allowing for the collection of the intruder's DNA onto the owner's hand (Table 12). It is also possible that the intruder's DNA that was detected on the owner's dominant hand was collected throughout the owner's occupation of the office space when contacting other surfaces that the intruder had touched previously. The intruders were excluded from the remaining owner hand samples (7/8).

Of the 8 samples taken from clothing, 1 sample from the back of the owner's shirt did not generate a profile and was excluded from further analysis. The owners were not excluded as sole, major, or majority contributors in all 7 samples (all LRs > $3E10^{6}$, av. of $2E10^{25}$). The intruders were excluded as contributors in all but one (LR=2) owner clothing sample. Live-in partners of office owners 3 and 4 were not excluded as minor contributors in 2 of the 7 samples (all LR > 800, av. of $8E10^{4}$) (Supplementary data 4). The results of the face samples have been published previously [37].

3.12.2. Intruder samples

The intruders were not excluded as the sole or major contributor in all 8 hand samples (all LRs > $1E10^{15}$, av. of $1E10^{26}$). The owners were not excluded as a minor contributor in 3 samples (all LRs > $1E10^{5}$, av. of $9E10^{18}$). Owner of office 2 was detected on both hands of the intruder in that office. It is noted that the last surface this intruder contacted before the hand sampling was the external doorhandle which was previously contacted by the owner when leaving after their first occupation of the office space (Table 13). The owner of office 3 was not excluded from the dominant right-hand sample of the intruder in that office. Again, the last surface contacted by both participants before sampling of hands was the external doorhandle (Table 13). The owners were excluded from the remaining 5 samples.

The intruders were not excluded as the major or majority contributor in 5 of the 8 clothing samples (all LRs >1, av. of $2E10^{26}$) and as a minor contributor in two of the samples (both LRs > $1E10^{7}$, av. of $1E10^{8}$). The intruder was excluded as a contributor in one sample. The owner was not excluded as a majority contributor in 2 of the clothing samples (both LRs >$2E10^{8}$, av. of $2E10^{16}$) and as a minor contributor in 3 samples (all LRs >700, av. of $3E10^{13}$). The owner was excluded as a contributor from 3 samples (Table 13, Supplementary data 4). During the occupation of the office spaces, for all offices and participants, the majority of time was spent by sitting in the office chair while performing office tasks. When sampling the office chairs, it was observed that only the owner's DNA was recovered, regardless of whether the intruder contacted the chair more frequently or for a longer duration of time. This detection of the owner's DNA on the office chairs (between 0.4 and 11 ng) may explain the detection of the owner on the intruder's clothing samples.

The results of the face samples have been published previously [37].

Table 12
Breakdown of DNA results obtained from the owner's personal samples in Category 9.

No. of samples	Sample Surface	Contributor			Av. DNA (ng)	
		Owner	Intruder	Associate	Owner	Intruder
1	Hand	Sole	Excluded	Excluded	3.2	0
5	Hand	Major	Excluded	Excluded	10.3	0
1	Hand	Major	Excluded	Minor	14.3	0
1	Hand	Major	Minor	Excluded	11.2	0.2
1	Clothing	Sole	Excluded	Excluded	0.3	0
1	Clothing	Major	Excluded	Minor	0.3	0
1	Clothing	Major	Minor	Excluded	2.3	0
1	Clothing	Majority	Excluded	Minor	0.4	0
3	Clothing	Majority	Excluded	Excluded	0.16	0

Table 13
Breakdown of DNA results obtained from the intruder's personal samples in Category 9.

No. of samples	Sample Surface	Contributor			Av. DNA (ng)	
		Owner	Intruder	Associate	Owner	Intruder
1	Hand	Excluded	Sole	Excluded	0	112.5
3	Hand	Excluded	Major	Excluded	0	0.8
1	Hand	Excluded	Major	Minor	0	3.1
3	Hand	Minor	Major	Excluded	1.1	16.4
1	Clothing	Minor	Major	Excluded	0.1	0.2
1	Clothing	Excluded	Major	Excluded	0	0.04
2	Clothing	Minor	Majority	Excluded	0.3	1.0
1	Clothing	Excluded	Majority	Excluded	0	0.08
1	Clothing	Majority	Minor	Excluded	0.4	0.3
1	Clothing	Majority	Minor	Minor	0.4	0.3
1	Clothing	Excluded	Excluded	Excluded	0	0

4. Discussion

4.1. General observations

DNA profiles were generated from 98 % of the samples collected within the four office spaces and the personal samples taken from the participants. The minimum number of contributors varied greatly within the office spaces, ranging from single source profiles to six person mixtures. Ninety percent of these DNA profiles were mixtures, with the owner of the office space not being excluded from 87 % of the samples. The owner was a sole or a major/majority contributor in 77 % of the samples. This high prevalence of the owners' DNA in the office spaces is not unexpected due to prolonged occupation of these spaces from 1 to 10 years. Similarly, a study by Breathnach et al. [38] investigated, among other factors, the probability of detecting the habitual wearer of a garment, detecting the owner in 51 % of the samples collected as a sole or major contributor. Atkinson et al. [8] found that the owners of "burglary" tools persisted as major contributors regardless of the subsequent use by one-time users. Such prolonged use of an item or location by an owner or usual user allows for the accumulation of the owner's DNA over time in quantities that can overwhelm relatively smaller quantities deposited during a short time use. However, the shedder status of the individuals contacting the surface is a contributing factor. Additionally, from video recordings it was noted that owners made more frequent and longer contacts with most items in this category. Perhaps office owners felt more comfortable in their own spaces, thus contacting more surfaces and for longer periods of time. Further, in the present experiment, several sampling categories were of the intruder contacts only; yet DNA of the owner was frequently detected, likely as background from previous use. To ensure that all the DNA transferred during contact was collected, samples taken during this study encompassed areas larger than the visualised contact; possibly collecting more background DNA than would have been present in the contacted area alone. In this experiment, the duration of the office occupation ranged from 1 to 10 years. It is not known after what duration of use the level of background DNA within office spaces plateaus/stabilises; if it is days,

weeks, months or over a year. Though one would expect this to have been reached in the offices used in the study, the level of background DNA may be less in a newly constructed/setup office space than in space used for a year or more.

In casework, many areas are sampled based on the assumed contacts and thus, as in the case in this study, also likely collect DNA from both contacted and additional surrounding background areas. The intruders were not excluded from 31 % of the samples. Intruder was the sole or the major/majority contributor in 14 % of the samples and was observed to contact these surfaces directly and/or more frequently than the owner during the experiment. The size of the items sampled may have also contributed to the detection of the intruder, as a small sample surface would have been more readily overwhelmed by the intruder's DNA during contact (e.g., pens), compared to larger items such as a chair seat. Future studies that systematically assess the effect of item size while keeping the other variables constant are needed to better understand their effects. Further, the indoor environmental conditions within the offices in this study, such as the indoor temperature and humidity, while deemed relatively constant (with all testing done within similar offices within the one complex, at the same time of year with similar indoor conditions) were not recorded. As studies show that environment can play a significant role in DNA transfer, persistence and degradation [2,39], in the future, the impact of different environments conditions such as temperature and humidity should be assessed.

While duration of contact did not significantly affect the amount of DNA detected, increased number of contacts resulted in significantly more DNA detected. Not surprisingly shedder status also played a role in the amounts and types of DNA profiles detected. High shedder was the owner of office 2 and this office, on average, had more DNA and resulted in more frequent detection of the owner compared to the remaining offices. Further, samples from this office had, on average, a lower number of contributors (2) compared to other offices. Similar findings have been noted previously, with high shedders tending to overwhelm the detection of traces from others that may have been present [35,40]. Incidentally, all the intruders in this study were low shedders, possibly resulting in lower detection of these participants in the selected samples. Further studies, that include participants with different shedder statuses, are needed to better understand how higher intruder shedder status may influence the results.

Unknown contributors were detected in most of the samples. Detection of trace unknown contributors on commonly contacted surfaces is an expected finding that has been noted previously [7,27]. It is possible that these unknown donors are brought into the office space on hands and personal items of the owners and visitors as well as being directly deposited by visitors for whom we did not have elimination DNA profiles. This latter contention is supported by the results of mixture-to-mixture analysis where common unknown donors were detected on several surfaces within each office space.

4.2. Categories 1–3: contacted by owner only

Samples in this category were all from the owners' offices and only touched by the owners. It is, therefore, not surprising that the owner was detected in all samples. Further, owners were detected as the sole/major/majority contributors, except for a whiteboard marker in office 4, where the owner was the minor and an unknown donor the majority contributor. The contact history of this whiteboard marker, prior to the experiment, is unknown but owner contacts during the study were infrequent and short in duration. Previous studies on handling of items by multiple people showed that shedder status, manner and duration of use are all relevant factors to the types of the DNA profiles that can be detected [17,20–23]. As the office owner of office 4 was determined to be a low shedder, it is possible that the unknown donor was either a previous user of the marker or indirectly transferred, for example by the hands of the low shedding owner. A close associate of the owner of office 1 was detected as a minor contributor, along with major contribution from the owner, on a whiteboard marker from that office. This individual visited the office space during the last hour of occupation contacting the chair and the desk surfaces, but not the whiteboard marker. The owner was observed using the marker once, for a total duration of 19 seconds. Again, it is possible that the close associate had used the marker prior to the experiment or came to be on this item through indirect transfer. The visitor was determined to be a high shedder, increasing the likelihood of their detection either from previous direct use, that persisted, or from indirect transfer. Several studies show that DNA from a first user can persist after subsequent use, especially if high shedders are involved [20,21,23,41]. Research is also available that demonstrate that normal, everyday social interactions can result in detectable indirect transfer [7]. Finally, a third possible means for the visitor's DNA to arrive on the marker is via contactless transfer. Contactless transfer refers to the transfer of biological material and DNA to an item without any physical contact. The visitor was observed to walk past the marker location, possibly shedding their skin cells and saliva aerosols to the target surface. Recent research shows that DNA can be present in the air for a period of time, dislodged and aerosolised from its initial deposit surface or shed from people, before depositing on nearby locations [30,42–44].

The intruders did not contact any of the items in this category and were excluded as contributors from all DNA profiles obtained. While there were possibilities for indirect transfer during the third hour of occupation, through surfaces and items contacted by both participants followed by contacts with "owner only" items, such transfers were not detected in the present study.

4.3. Category 4: contacted by intruder only

In this category, the office owners were detected as sole, major or majority contributors in 72 % of samples, even though they did not contact these items during the observation period. This background DNA is likely a consequence of the previous use of the space by the owners resulting in DNA accumulation on commonly used surfaces. In contrast, the DNA of an intruder was detected in 41 % of directly contacted samples with increased number of contacts resulting in higher level of detection. In general, where the intruder was detected as a major contributor, a review of the recordings showed that intruders made more contacts and for longer duration than the owners that were detected as minor contributors. Interestingly, intruders were excluded from 59 % of the samples that they contacted, sometimes multiple times, immediately before sampling. van Oorschot et al. [3], in review of the literature noted that direct contacts did not always result in detection which, along with the results of this study, highlights that not every contact leaves a detectable trace.

One sample of note in this category is the chocolate bar wrapper where the owner, a high shedder, was detected as the minor contributor. This chocolate bar wrapper was cleaned and introduced into the office space DNA-free at the beginning of the second hour and was not contacted by the owner. The intruder ate the chocolate bar and placed the wrapper in the bin. The hands of the intruder had contacted surfaces within the office prior to contacting chocolate bar wrapper, so may have picked up some of the owner's DNA during these actions and subsequently transferred some of it to the wrapper. Additionally, and/or alternatively, whilst eating the chocolate bar, it was placed, in its wrapper, on a desk surface and from this action, the DNA of the owner may have transferred onto the chocolate wrapper. It is also possible that the owner's DNA was present within the drawer or the bin where the chocolate bar was initially located. Reither et al. [45] showed that DNA can transfer from benches, drawers and table surfaces to the items located within or on top.

4.4. Categories 5, 6 and 7: contacted by owner followed by intruder; contacted by intruder followed by owner; and contacted during all three occupations

Office owners were detected as either sole or major contributors and intruders were excluded in all samples in the "owner followed by intruder" category. Of note, while intruders made the last contact with all items in this category prior to sampling, owners were noted to make longer contacts overall. Several studies show that longer contacts can result in higher accumulation of the subject's DNA, overwhelming donors who made shorter or less frequent contacts [2,7,46–50]. Most of these items, such as the phone and the computer, are known to be in routine use by the office owners and thus accumulate their DNA. A study by Fonneløp et al. [22] showed that DNA of a regular user of a computer, a keyboard and a mouse can persist for up to 8 days after use by a second individual. However, in contrast to our results, in the Fonneløp study, the secondary user was detected as a contributor in some of the samples. Factors such as duration of use, number of contacts or the shedder status of the tested individuals may have contributed to these differences. In our study all intruders were determined to be low shedders. These results highlight that the last contact will not always be the determining factor for detection of somebody's DNA and factors such as history of use and shedder status may influence the change of detection in some circumstances.

For the "intruder followed by owner" category it was hypothesised that there would be a wide variety of relative DNA contributions, but intruders would be detected less frequently than the owner due to the latter being the last to contact the surface. In this set of samples, the owner was detected, usually, as the sole/major/majority contributor, while the intruder was excluded from most of these samples. It is possible that contact by the owners prior to the experiment, resulted in their greater detection.

Consistent with the results from category 5 "owner followed by intruder" items in category 7 that were contacted during all three occupations (i.e., owner, followed by intruder, followed by owner), the owner again was detected in all samples that produced profiles (100 %) while the intruder was excluded (63 %) highlighting the significance of previous contacts and background DNA in personal spaces.

4.5. Category 8: not contacted by anyone during the experiment

While items in this category were not contacted during the experiment, it is likely that the owners would have contacted these surfaces at some time prior to the recorded activities. Meakin et al.[51] showed that a regular user can still be detected over a week after a surface has been contacted. These findings correspond to the results of the present study, where an owner's DNA, from previous use, was detected in 72 % of the samples; and align with the observations regarding background in categories 1–7.

An interesting result in this category is the drawer handle in office 2 where the intruder was detected as a minor contributor. This result was unexpected as neither direct nor indirect transfer, based on video recordings, could explain this result. The cabinet drawer was not touched by the owner or intruder during the duration of experiment. The intruder was observed to walk near the item when occupying the office space. It is possible that the action of walking agitated the intruder's clothes enough to dislodge DNA, or otherwise shed their DNA to the environment, and allow contactless transfer to the cabinet drawer [30,42–44,52].

4.6. Category 9: hands and clothing

As expected, [53–55], hand donors were always detected in their own hand samples, as sole/major/majority contributors. The non-donor participants (owners or intruders) were detected as minor contributors in 50 % of these samples. The intruders and owners had no direct contact at any time prior to or during the experiment, thus these results represent indirect transfer events. Not surprisingly, owners were detected more frequently on the hands of the intruders due to the higher prevalence of owners DNA in their personal office space. The owner of office 2 was not excluded as a minor contributor on both intruder's hands. This is possibly due to the high shedder status of the owner resulting in large amounts of DNA being detected on the surfaces in this office and available for collection by the intruder. Similarly, Samie et al. [56] found that 97 % of their plastic knife handle samples detected foreign DNA from activities participants performed in a shared space.

Clothing wearers are routinely found on their personal garments and the type of profile detected is influenced by sample's location (inner or outer surfaces) and contacts with the skin [21,23,41,47,57–61]. In our study, the wearer was detected in 97 % of the samples. The one sample where the wearer was excluded was from the back of the office 1 intruder's shirt. When clothing is worn, only the inner surface of the garment touches the skin and is therefore more likely to collect the wearer's DNA. The outer surfaces of the garments are exposed to the environment and can accumulate DNA from various other sources. Further, this intruder was determined to be a low shedder and was not observed touching their clothing during the experiment, possibly explaining the absence of the wearer's DNA [47]. Office owners were detected on five intruder clothing samples (63 %), two of which resulted in mixture inversion where the owner was detected as a majority contributor although they did not wear these clothes. These results represent indirect transfer events, also noted in other studies, where intruder's clothing picked up DNA of the owner from office surfaces, such as a chair [25,59,62].

The DNA of live-in partners of the owners were detected in 29 % of the owners' clothing samples. These samples were from the backs of the shirts from two separate office owners. Cohabitating partner's DNA can be deposited during direct contacts as well from washing, drying, ironing, or folding shirts [47]. Stouder et al. [63] found that clothing, when worn for a day after laundering, contained DNA from the wearer's spouse. While Magee et al. [58] investigating the contributions of self and non-self DNA to clothing, observed the spouse of the wearer of a garment in 75 % of the samples.

4.7. Comparison to office occupation study by Goray et al. [27]

Comparisons were only made to the results from Laboratory A within the study by Goray et al. [27] as samples from this laboratory were processed using similar methods to those employed in the present study. This study yielded overall lower DNA amounts when compared to the results of the previous study by Goray et al. [27], that investigated DNA amounts transferred by an intruder to the single occupation office space. The amounts of DNA in Goray et al. were found to be between 0 ng – 220 ng (average 15.1 ng), while amounts detected in the present study ranged between 0 ng – 122 ng (average 6.7 ng). The difference in the overall DNA yields may have been due to the difference in duration of the experiments. The Goray et al. study was conducted over one workday, lasting approximately six hours, whereas the current study was conducted over three hours. However, this may be unlikely given that the offices in both studies had been in continuous occupation for several years, unless the shedder status of the intruders was much higher than that of the owners. The shedder status of the participants in this study (most were found to be low shedders) may be a contributing factor. No shedder testing was done in the previous study, thus precluding direct comparison of the shedder influence on both sets of results. Both studies found that the highest yields, in general, were from high use items such as phones, keyboards, and the office chair samples. Conversely, both studies obtained lower DNA amounts from the light switches, possibly due to their small contact areas.

In Goray et al. [27], the prevalence and detection of the owner's and intruder's DNA was 92 % and 40 % respectively. The present study generated comparable numbers with owner and intruder detected in 7 %

and 31 % of samples respectively. It was reported in the Goray et al. study that the temporary occupant was able to be detected on all the pen samples as the majority or major contributor when it was known that a pen was used by the occupant between 30 and 120 minutes [27]. Similarly, the current study found that the intruder was detected on two of the four pens they were observed to use. Somewhat lower detection of the intruder in this study may be due to shorter use of the pens as well as the reoccupation of the office space by the owner, which was not part of the original study. Pfeifer and Wiegand [23] when investigating the persistence of DNA on tools used in a burglary scenario, found that the DNA of the previous user was removed and/or overwhelmed by a subsequent user. Unknown contributors were also detected in most samples in both studies (75 % and 85 % of Goray et al. [27] and present study samples, respectively), an expectant occurrence with touched surfaces.

Finally, there were several instances of possible indirect transfer theorised by Goray et al. [27], based on their results and the use of questionnaires that documented activities performed by the participants in that study. This study was able to confirm several indirect transfer events, through review of every contact made by both hands of each participant during their 3 hours of occupation.

5. Conclusion

The transfer, prevalence and persistence of DNA and the variables that influence these factors were explored within four office spaces. The DNA of the owners was more prevalent than of the intruders in all office spaces likely from background DNA accumulation. Direct contact resulted in detectable DNA transfer in 55 % of the samples; while indirect transfer was detected in 6 % of the samples, and contactless transfer in 0.5 % of the samples. However, it should be acknowledged that the actual number of indirect transfer events may be underestimated, as owners' background DNA may have masked some of these events. The 45 % of contacts that did not result in the detection of the participants may have been due to the low frequency and/or duration of contact with the item, low shedder status of many of the participants involved, post contact removal by subsequent contacts (relevant to some categories) and presence of background DNA. It was also found that the last person to contact an item or surface was not always the major contributor in 18 % of the samples.

A significant increase in relative DNA contribution was observed with increased number of contacts while the shedder status of the individuals was not observed to have a significant impact on the amounts of DNA detected. Of note, most of the participants in this study were low shedders and thus insufficient number of high and intermediate shedders may have been present for proper assessment of the shedder influence on the DNA results. Further, some of the impacts of the duration and number of contacts, especially for owners, may have been masked by the presence of background DNA. As this study attempted to replicate real-life circumstances, it is likely that all variables recorded in this study (i.e., number and duration of contact, shedder status, and contact history) and other variables such as the surface type, porosity, and environmental conditions all had an impact on the results collected.

Overall, the results of this study show that regular occupants and users are the most prevalent donors to the biological material detected, but also highlights that a relatively short single occurrence occupation can result in detection of that person, sometimes as a major or single contributor, even after brief reoccupation by the regular users of the space. Further, indirect and contactless transfer events while not as common as direct contact transfers, do occur and can be readily detected. This data may assist forensic practitioners in targeting different spaces, based on whether they are targeting usual occupant or an intruder as commonly used surfaces are more likely to have owner's DNA in quantities that can overwhelm short contact transfer. Finally, this data can also assist with activity level evaluations where spaces known to be occupied by small number of people are being investigated.

CRediT authorship contribution statement

Oliva Handt: Writing – review & editing, Supervision, Project administration, Methodology, Formal analysis, Conceptualization. **Mariya Goray:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Monique Zacher:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Roland A.H. van Oorschot:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization.

Declaration of Competing Interest

The authors declare no competing interests.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.fsigen.2024.103130.

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