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Short Communication

Comparison of DNA recovery methods and locations from regularly-worn hooded jumpers before and after use by a second wearer

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ABSTRACT

Items of worn clothing are routinely examined for DNA in forensic casework, commonly with the expectation that at least some of the DNA will come from a wearer of the item, so-called 'wearer DNA'. This study investigated DNA recovered from hooded jumpers that were regularly worn and laundered for four weeks and then subsequently worn by a different individual for four hours. This study also systematically investigated whether using different recovery methods or sampling locations on the jumpers might distinguish between DNA deposited by the regular and most recent wearers of clothing. Four volunteers each wore a new hooded jumper regularly (6 h/day, 2 days/week, washed at weekends) during two 4-week periods. At the end of each month, DNA was first recovered by cutting out and mini-taping the inside left cuff, half-collar, pocket and underarm fabric. The jumpers were then worn by a different individual for four hours, and DNA was again recovered by cutting out and mini-taping, but this time from the inside right cuff, half-collar, pocket and underarm fabric. All DNA samples (n = 128) were quantified and profiled. DNA quantities ranged from 0 to ~40 ng with an outlier of ~150 ng, and no significant differences were observed among recovery methods and sampling locations, nor whether one or two wearers had worn the jumpers. However, one volunteer consistently deposited significantly more DNA to their jumpers than two other volunteers, confirming the impact of 'shedder status' on DNA deposition during wearing of clothing. When jumpers were regularly worn by one wearer, the majority (72.7–83.3 %) of the samples for all wearers across both months comprised a major profile of the wearer with a minor profile of non-wearer alleles. When jumpers were then worn by a second wearer, the composition of the profiles obtained were generally reproducible across the recovery methods used, the sampling locations and the two replicates of the experiment for each pairing of wearers. However, profile compositions differed between wearer pairings. Overall, ~60 % of profiles obtained gave a major profile of the regular wearer, whereas ~30 % gave a major profile of the second wearer. The remaining profiles comprised other much less frequent observations of single-source profiles of each wearer and equal proportions of DNA from both wearers. Non-wearer DNA was also observed in the majority of samples, both before and after jumpers were worn by a second wearer. For one volunteer's jumpers, a recurring non-wearer DNA profile was observed that could be attributed to their romantic partner, and this DNA persisted on the jumpers even after being worn by the second wearer. This study provides insight on the impact of shedder status, multiple wearers, different recovery methods and sampling locations on the quantities of DNA and compositions of DNA profiles recovered from authentically regularly-worn hooded jumpers. The findings also provide a preliminary dataset that can be used to infer activity level probabilities in casework.

1. Introduction

In many countries across the world, items of clothing are routinely examined for DNA in forensic casework [1–9]. Such items are examined with the expectation that at least some of the DNA will come from a wearer of the item, so-called 'wearer DNA'. However, with the increased

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sensitivity of DNA technologies in the last decade, the prevalence of recovering mixed DNA profiles from items of clothing has increased. As such, issues have been raised within the published literature as to what exactly does 'wearer DNA' mean as a term [1,10,11]. Does the DNA recovered come from a wearer of the item, or from a handler of the item, or was the DNA indirectly transferred to the item? And if the DNA was indeed deposited via wearing, did the DNA come from the regular (alternatively referred to as habitual or usual) wearer of the item, or someone who has worn the item more recently? It is our experience that attempting to answer these questions can be debated within casework when evaluating the DNA profiles obtained from clothing exhibits. As a result of such debates, van Oorschot *et al.* [12] emphasise that using the term 'wearer DNA' could be misleading, since it implies that the DNA was deposited by the action of wearing, which might not be the case.

When considering the evaluation of a DNA profile from a mixture given propositions regarding activities that resulted in DNA deposition, it has been proposed that the relative proportions of DNA within such a mixture may be informative, for example, with respect to distinguishing handlers of an item [13]. Therefore, if a mixed DNA profile recovered from worn clothing can be deconvoluted into major and minor profiles, then this might be informative in evaluating the DNA profiles given relevant activity level propositions (i.e. propositions addressing regular wearing of the item, most recent wearing prior to retrieval of the item, or other activities that resulted in direct or indirect DNA transfer). If it is accepted that the major DNA profile recovered came from a wearer of the item (an assumption that may or may not be appropriate, depending on the circumstances), then is that wearer the regular or most recent wearer? There are conflicting statements within the literature on this, for example, Taupin & Cwiklik [14] states "*The usual wearer of a garment should be detected as the major source of DNA on a garment…*", whereas Breathnach *et al.* [15] states "*Some research on trace DNA suggests the last user/toucher results as the major contributor to mixed DNA profiles. This has been observed on clothing…*". A study by Stouder *et al.* [7] is referred to in order to support this latter statement, but that study did not include multiple wearers, so no inference can be made regarding the most recent wearer (as discussed in [10]). Furthermore, we have observed statements in forensic casework reports, such as this from a UK case in 2015, "*A major profile matching* [the defendant] *is what I would expect if he was a regular/usual wearer of the jacket*". These statements on whether the major DNA profile from a worn item of clothing can be attributed to the regular or most recent wearer illustrate the magnitude of this issue, especially if the available research does not support such inferences.

In recent years, there have been a number of studies starting to address the nature of the DNA profiles obtained from items that have been worn by more than one person. An initial study, in which various items of clothing, including baseball hats, T-shirts and sweatpants among others, were worn by the regular wearer for several hours or overnight and then worn by a second wearer for about one hour, showed that mixtures of DNA from both wearers, plus additional indirectly-transferred sources, were recovered by all DNA sampling methods used [16]. More recent studies on investigating multiple wearers have used T-shirts [3] and accessories, including gloves [3,17–19], nylon/polyester bracelets worn on the arm [20], nurse caps worn on the head [17,18] and cotton sweatbands worn on a non-defined area, presumably arm or head [21]. These studies have taken a more structured approach to experimental design, in which participants wore/used the items in a prescribed manner to simulate DNA deposition during regular wearing and then different participants wore the items for set timeframes as the most recent wearers. Dziak *et al.* [3] did not report the DNA contributions from the respective wearers for the samples obtained from the T-shirts, presumably due to too few samples yielding DNA quantities above their amplification threshold, but they did report a range of mixed DNA profiles from the worn gloves. These included samples with equal proportions of DNA from both wearers, samples with major DNA profiles from the regular wearer, and samples with major DNA profiles from the most recent wearer [3]. For the other studies, it was generally observed that with shorter durations of wear by the most recent wearer (several minutes to hours), the items yielded major DNA profiles from the regular wearer, but items worn for longer durations by the most recent wearer (several hours to days) yielded major DNA profiles from the most recent wearer [17,18,20,21]. In particular, Poetsch *et al.* [21] observed that 10 min of wearing of sweatbands by the second wearer was sufficient to recover their complete DNA profile in > 75 % of samples, and that a complete or partial DNA profile from the second wearer could always be recovered, irrespective of the durations of wear by either wearer [21].

Given the variability in the DNA mixtures obtained from items worn by multiple individuals, it is evident that it is not as clear cut as the major DNA profile coming from the regular or most recent wearer. A review by Meakin & Jamieson [10] of the primary research on this topic led to the conclusion that it is not possible to infer which individual last wore an item from the DNA profile recovered, and that the respective proportions of DNA obtained depend on a range of factors, including, for example, duration of wear, type of substrate, and 'shedder status'. However, it has been suggested that using different methods to sample items of clothing might allow differentiation between DNA profiles from the regular and most recent wearers [16]. In particular, Harris *et al.* [16] hypothesised that use of Gel-Pak '0′, a gel film that shares similar properties to adhesives and tends to recover the top layer of loose particulate, might recover single-source DNA profiles of the most recent wearer from clothing worn by multiple wearers. Whilst their data did not fully support this hypothesis, they did observe that Gel-Pak '0′ and swabbing tended to recover more DNA from the most recent wearer, whereas scraping (a more invasive method) tended to recover more DNA from the regular wearer [16]. Therefore, the first aim of our study investigated whether, of the two methods routinely used to recover DNA from clothing in UK casework, mini-tapes (adhesives) would recover more DNA from the most recent wearer and cutting out fabric swatches (an invasive method) would recover more DNA from the regular wearer.

The study by Dziak *et al.* [3] also investigated whether the method used to sample DNA from worn clothing could shed some light on the respective wearers, along with considering whether the location sampled could also be informative. Their DNA profiles from the gloves worn by multiple wearers showed varying results. Swabbing yielded 9/20 DNA profiles in which the major profile came from the most recent wearer and none of the DNA profiles gave a major profile of the regular wearer, whereas cutting out 1 cm^2 swatches yielded 11/20 DNA profiles with a major profile from the regular wearer, but also 7/20 with a major profile from the most recent wearer [3]. Although varied, these results also suggest that there is scope to investigate the impact of different recovery methods on the DNA profiles obtained. In addition, Dziak *et al.* [3] considered the impact of sampling location on DNA recovery success rate, targeting inside collar and underarm of the worn T-shirts and various areas of the worn gloves. No statistically significant differences in recovery success rate were observed among the different locations for both types of item [3], but no detailed analysis of any potential impact of sampling location on composition of the DNA profiles obtained was provided. As such, the second aim of our study was to investigate whether different sampling locations could consistently and reproducibly yield DNA profiles of differing compositions of DNA from the regular and most recent wearers.

To address these two aims and assess whether a sampling strategy employing different methods and/or locations could help distinguish between DNA profiles from different wearers of an item, a structured experiment was set up using brand new hooded jumpers that were worn and laundered by participants over four weeks to become 'regularly worn'. Samples for DNA quantification and profiling were taken by mini-taping and cutting out swatches from various locations on one half of each hooded jumper. The hooded jumpers were then worn for four consecutive hours by a different wearer and the same locations on the other half of each hooded jumper were sampled using the same methods. Sampling in this manner not only allowed comparison of the DNA profiles obtained among different methods and locations, but also

comparison of DNA profiles obtained before and after the hooded jumpers were worn by the second wearer. This experiment was conducted with four pairs of participants and was repeated twice across two consecutive months to assess the reproducibility of the results obtained, and therefore increase the value of these results in furthering our understanding of the contributions of DNA from different wearers on an item of clothing.

2. Materials and methods

2.1. Materials and volunteers

Eight new grey hooded jumpers (material: 65 % polyester, 35 % cotton) were purchased from Primark and stored in the laboratory until ready for use. Volunteers were recruited for this research project in accordance with ethics approval granted by UCL's Research Ethics Committee (ref. no. 5107/001) and all those who participated gave informed consent. Four volunteers (V1-V4) participated who were available for the two-month period and willing to wear the provided hooded jumpers for the set time periods and record their activities. The volunteers were three females and one male, all aged 25–30 and with no known skin conditions. Table 1 shows whether the volunteers lived with others and how they laundered and stored the provided hooded jumpers when not in use. The participants and the romantic partner of V4 each provided a buccal swab from which a reference DNA profile was generated.

2.2. Experimental design

Each participant wore their provided hooded jumper regularly for four consecutive weeks. To maintain a level of consistency in the wearing of the hooded jumpers across the four participants, a wearing schedule was provided as follows. During each week, each volunteer wore their hooded jumper on Monday and Wednesday, and then washed the hooded jumper at the weekend along with their other laundry and according to their normal washing protocol (Table 1). The hooded jumper was worn for a total of six hours per day (unless otherwise stated), which could be consecutive or intermittent; this was left undefined so that the volunteers could wear the hooded jumper as they would naturally, i.e. taking it off and putting it on as they wished. On the fourth week, participants delivered their hooded jumpers to the laboratory on the Thursday, after their two days of wear and before laundering, and samples were taken for DNA analysis from locations on the left-hand side of each hooded jumper (Fig. 1). On the Friday, each volunteer returned to the laboratory and collected a different hooded jumper from their own. They wore that hooded jumper for four consecutive hours, defined in order to represent the use of a borrowed or stolen hooded jumper during a crime activity, and then returned the hooded jumper to the laboratory and samples were taken for DNA analysis from locations on the right-hand side of the hooded jumper (Fig. 1).

Timings for delivery and collection of hooded jumpers were staggered by 30 min to prevent potential interaction between participants. Whilst awaiting DNA sampling and/or collection by the participants, the hooded jumpers were stored individually in brown paper bags, in accordance with the London Metropolitan Police evidence packaging procedures. For each half of a hooded jumper (left or right-hand side as stated above), samples were taken from the inside surfaces of the cuff, base of the pocket, underarm fabric and half of the collar (Fig. 1). Samples were taken from each location by first cutting out a section of approximately 1.5 cm x 0.5 cm using DNA-free scissors or scalpel, and then taping the remainder of the location using Scenesafe FAST™ mini-tapes (SceneSafe^{TM}, UK) as per the manufacturer's instructions. The approximate sizes of the areas taped were: 0.9 cm x 25 cm along half the inside collar surface, 0.9 cm x 22 cm along the inner surface of one whole cuff, 0.9 cm x 15 cm along the base of the pocket, and 3 cm x 3 cm covering the seams of the underarm fabric. The cut-out sections (n = 32) and mini-tapes (n = 32) were processed for DNA extraction, quantification and profiling, as described in Section 2.3.

The four-week experiment was repeated with new hooded jumpers,

Table 1
Details regarding whether the participants lived with others and the washing and storage conditions they used for their respective hooded jumpers.

Volunteer	Lives…	Washing conditions	Storage conditions
V1	With 5 other unrelated people	• 40 °C, bio detergent, fabric conditioner • Washed and air dried only with own clothes • Communal washing machine shared with ~ 40 others	On chair at work in communal office or on chair in bedroom
V2	Alone	• 30 °C, bio detergent, fabric conditioner • Washed and air dried only with own clothes • Communal washing machine shared with ~ 15 others	In wardrobe with other clothes
V3	Alone	• 40 °C, non-bio detergent, no fabric conditioner • Washed and air or tumble dried sometimes with clothes from one other individual • Communal washing machine shared with ~ 15 others	In wardrobe with other clothes, sometimes stored with clothes of one other individual
V4	With romantic partner	• 30 °C, bio detergent, fabric conditioner • Washed and air dried with clothes of romantic partner • Own washing machine	In wardrobe with other clothes

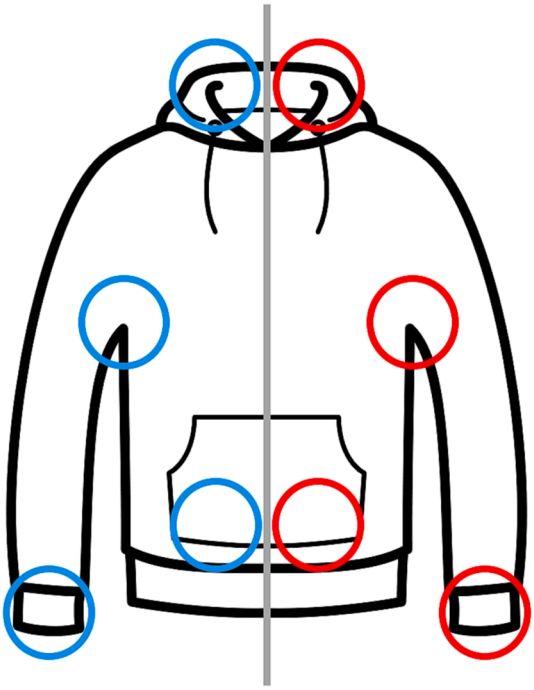

Fig. 1. A schematic of a hooded jumper to illustrate the locations that were sampled after the regular wearer had worn the jumper (blue) and those sampled after the second wearer had subsequently worn the jumper (red). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

giving a total of 128 samples for DNA analysis. The two replicates of the experiment were conducted in two consecutive months in the UK. Participants were paired as 'regular wearers' and 'second wearers' according to size to allow them to be able to wear the same sized hooded jumpers; hooded jumpers worn regularly by V1 were then worn by V3 (V1:V3) and vice versa (V3:V1), and hooded jumpers worn regularly by V2 were then worn by V4 (V2:V4) and vice versa (V4:V2). Participants were asked to keep a record of their activities and interactions with others whilst wearing the hooded jumpers. These records were analysed semi-quantitatively using content analysis; where participants had not specified the precise time of certain activities, inferences were made according to previous records from the same participant. Whilst the activities of the participants during their regular wearing of the hooded jumpers are presented in Section 3.2, the activities during the second wearing of hooded jumpers were very similar across the four participants and the two months. For both months, both V1 and V3 wore the hooded jumpers whilst in a communal office and/or kitchen for the full four hours, and V2 spent 15 min walking and then the remaining time in a communal office and/or kitchen. For V4, they wore the hooded jumper in a communal office and/or kitchen for the full four hours in month 1, but only for 2.5 h in month 2, with the other 1.5 h in a laboratory wearing a laboratory coat over the hooded jumper.

2.3. Processing of DNA samples

For DNA extraction of the hooded jumper samples, the mini-tapes were cut into strips with DNA-free scissors and processed using the QIAamp® DNA Investigator Kit (QIAGEN, Germany), as per the manufacturer's swab protocol. To maximise the DNA concentration obtained, 1 mg carrier RNA was added to the extraction process and DNA was eluted into 35 μl of Buffer ATE; extracts were then stored at −18 °C until required. The SwabSolution™ Kit (Promega, USA) was used to crudely extract DNA from the buccal swabs, as per the manufacturer's instructions. DNA quantification was conducted using the Quantifiler® Human DNA Quantification Kit (Applied Biosystems, Thermo Fisher Scientific, USA) with the QuantStudio™ 6 Flex Real-Time PCR System (Applied Biosystems, Thermo Fisher Scientific, USA), as per the manufacturer's instructions. DNA extracts from each sample were quantified in duplicate to enhance accuracy; averages of these duplicate quantifications are given in Supplementary Table S1. Samples were profiled using the AmpF*l*STR® NGM Select™ PCR Amplification Kit (Applied Biosystems, Thermo Fisher Scientific, USA) following the 30-cycle PCR protocol, and with the DNA Analyzer 3730xl (Applied Biosystems, Thermo Fisher Scientific, USA) using injection conditions of 3 kV and 5 sec (as per the laboratory's internal validation study). For the PCRs, depending on the quantification results of the DNA extracts, either 10 μl aliquots of undiluted extracts, or dilutions of extracts to 1 ng DNA in 10 μl, were amplified in 25 μl reactions. DNA profiles were generated and analysed using GeneMapper® *IDX* v1.3 software (Applied Biosystems, Thermo Fisher Scientific, USA) with a peak height threshold of 100 relative fluorescence units (rfu), in accordance with the laboratory's internal validation study.

2.4. Data analysis

Total quantities of DNA recovered in each sample were calculated by multiplying the concentrations obtained by the elution volume of 35 μl. Relative contributions of DNA as percentages from the regular wearer, second wearer and any non-wearer sources, including the romantic partner of V4, to the profiles obtained from the hooded jumpers were determined by comparison to the reference DNA profiles of participants and V4's partner. These calculations used the relative peak height contributions from the unique alleles that could be attributed to each of the respective reference profiles at each locus, and averaged across the STR loci [22]. To ensure the accuracy of determining these relative contributions, DNA profiles were only interpreted when at least one allele was observed at all loci, i.e. there was no locus drop-out. Minimum numbers of contributors were determined in two ways; first based on consideration of both number of alleles and respective peak heights, and second informed by the contributors identified by comparison to the relevant reference profiles. Datasets were statistically analysed using IBM SPSS Statistics version 28, with all data points included in the calculations. Differences were considered statistically significant when p values were less than 0.05. Effect size was only calculated for significant differences that were reproducible across both months of the experiment, and was interpreted from r^2, determined by squaring the value of r, calculated from $r = Z/\sqrt{N}$, where $N = 16$ for each comparison. Effect sizes were interpreted using the benchmark r (r^2) values of 0.2 (0.04) for small, 0.5 (0.25) for medium and 0.8 (0.64) for large [23], with the r^2 values presented here to provide an indication of percentage of explained variance.

3. Results

3.1. Effect of recovery method, sampling location and 'shedder status' on quantity of DNA recovered from worn hooded jumpers

The DNA quantification data for all 128 samples are presented in Supplementary Table S1 and show that the quantities of DNA recovered from the hooded jumpers varied across the recovery methods and locations sampled. Mann-Whitney U tests comparing the quantities of DNA recovered by cut-outs versus mini-tapes (Supplementary Table S2) showed no significant differences for hooded jumpers worn by both one and two wearers, with one exception. A significant difference in DNA quantity between cut-outs and mini-tapes was observed for V2′s regularly worn hooded jumpers for month 1 ($U = 0.0$, $p = 0.029$), but this was not replicated in month 2 ($U = 3.0$, $p = 0.15$). To examine whether the sampling location affected the quantity of DNA recovered, a Kruskal–Wallis test was employed to compare the effect of sampling the underarm, collar, cuff or pocket on the quantities of DNA recovered from the hooded jumpers. No statistically significant differences among the DNA quantities recovered were observed from the different sampling locations, irrespective of whether the hooded jumpers were worn by one or two wearers (Supplementary Table S2). It might be expected that hooded jumpers worn by two wearers rather than just one wearer would have an increased amount of DNA present. However, significantly more DNA from hooded jumpers worn by two wearers rather than one was only observed for V2′s regularly worn hooded jumpers for month 2 ($U = 8.0$, $p = 0.01$) and V3′s regularly worn hooded jumpers for month 1 ($U = 0.0$, $p = 0.001$), but neither of these results were replicated in the other month of wearing (Supplementary Table S2).

Given that the recovery method used and the location sampled did not reproducibly affect the quantities of DNA recovered, the quantities were combined across these variables to allow a comparison of the DNA deposited by different participants on their hooded jumpers, prior to use by the second wearer (Fig. 2). In month 1, V4 deposited significantly more DNA than V1 ($U = 2.0$, $p = 0.002$), V2 ($U = 0.0$, $p = 0.001$, $r^2 = 0.71$) and V3 ($U = 0.0$, $p = 0.001$, $r^2 = 0.71$). However, in month 2, V1 deposited significantly more DNA than during month 1 (Wilcoxon Signed Ranks Test $Z = -2.5$, $p = 0.012$, $r^2 = 0.40$), such that the quantity of DNA deposited by V4 only remained significantly higher than that deposited by V2 ($U = 9.0$, $p = 0.016$, $r^2 = 0.36$) and V3 ($U = 11.0$, $p = 0.027$, $r^2 = 0.30$).

3.2. DNA deposition on hooded jumpers through regular wearing

The respective proportions of DNA contributed by the regular wearer and non-wearer sources for each hooded jumper that had been regularly worn by a single participant are shown in Fig. 3. As with the quantities of DNA recovered, no apparent differences were observed in the composition of the DNA profiles obtained between the two sampling methods employed, or among the locations sampled, for all hooded jumpers

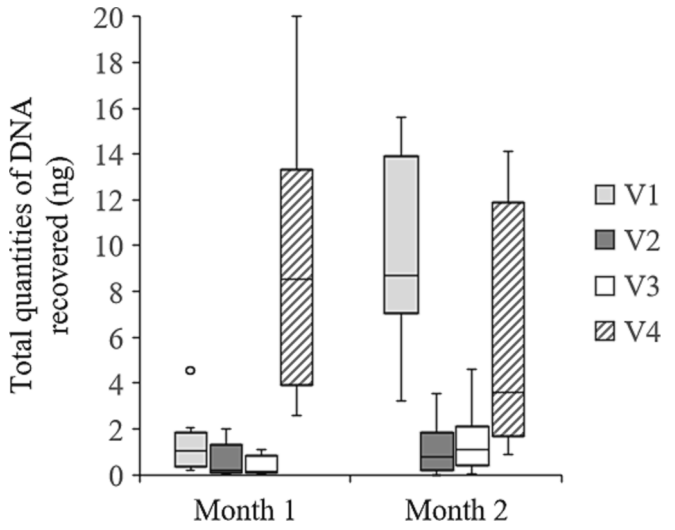

Fig. 2. Total quantities of DNA recovered from hooded jumpers regularly worn by each participant. Samples are combined from the different sampling methods (cut-out and mini-tape) and locations (underarm, collar, cuff and pocket) for each volunteer: V1 (light grey), V2 (dark grey), V3 (white), V4 (diagonal lines). For ease of visualisation, the 39.9 ng DNA quantity recovered from the collar of V4′s hooded jumper in month 2 using a mini-tape is excluded from this graph.

(Fig. 3). The activities of those participants whilst wearing the hooded jumpers are shown in Fig. 4. For wearer V1, even though their activities whilst wearing the hooded jumpers varied between months (Fig. 4(a) and (b)), all of their samples showed their DNA as either single-source DNA profiles (18.8 %; 3/16) or major profiles (81.3 %; 13/16) with minor DNA profiles from non-wearer sources (Fig. 3(a) and (b)). A similar observation was seen for wearer V2 (Fig. 3(c) and (d); Fig. 4(c) and (d)), for which all of their amplifiable samples showed their DNA as either single-source DNA profiles (16.7 %; 2/12) or major profiles with minor non-wearer DNA (83.3 %; 10/12). Four of the 16 samples failed to produce sufficient alleles to determine relative contributions of DNA due to the stochastic effects occurring at such low quantities of DNA (≤0.18 ng total DNA; Supplementary Table S1).

For the other two wearers (V3 and V4), more varied results were observed that showed occasions where their DNA was the major, co-major or minor contribution to samples from their respective hooded jumpers. For wearer V3, due to low levels of DNA on their hooded jumpers (Fig. 2; Supplementary Table S1), five samples did not produce sufficient alleles to determine relative contributions of DNA (Fig. 3(e) and (f)). Of the eleven samples that produced profiles across both months for wearer V3, two samples (18.2 %) gave approximately equal proportions between the wearer and non-wearer, with the other samples showing a single-source (9.1 %; 1/11) or major DNA profile (72.7 %; 8/11) from the wearer (Fig. 3(e) and (f)). The activities of this wearer were very similar for both months (Fig. 4(e) and (f)). For wearer V4, one sample (cut-out of collar in month 2) produced sufficient DNA (4.35 ng) for a profile, but did not produce a profile, even after re-analysing a dilution of the sample (Supplementary Table 1). For this wearer, a recurring distinctive non-wearer profile was observed and found to be from their romantic partner with whom they lived. Whilst the wearer's DNA was the major profile in most samples obtained (73.3 %; 11/15), the partner's DNA was the major profile for one sample (6.7 %) and in approximately equal proportions to DNA from the wearer in the remaining three samples (20.0 %; Fig. 3(g) and (h)). Transfer and persistence of the partner's DNA on the hooded jumpers was therefore observed in both months, even though the activities of the wearer whilst wearing the hooded jumpers varied between both months (Fig. 4(g) and (h)).

3.3. DNA recovery from hooded jumpers after being worn by a second wearer

Of the 64 samples from hooded jumpers worn by two wearers, only 60 samples gave interpretable DNA profiles (Supplementary Table S1); a summary of wearer and non-wearer contributions to these DNA profiles is shown in Table 2. Of these profiles, 61.7 % (37/60; 19 for month 1 and 18 for month 2) showed a major profile of the regular wearer, whereas 30.0 % (18/60; 10 for month 1 and 8 for month 2) showed a major profile of the second wearer (Table 2). The remaining profiles were comprised of one (1.7 %) single-source regular wearer profile observed in month 1, one (1.7 %) single-source second wearer profile observed in month 2, and three profiles (5.0 %; one in month 1 and two in month 2) in which the DNA from the regular and second wearers were observed at approximately equal proportions (Table 2).

When hooded jumpers worn regularly by V1 were then worn for four consecutive hours by V3 in month 1 (Fig. 5(a)), DNA from the regular wearer was observed as a single-source DNA profile for the pocket cut-out, and as the major profile with the second wearer's DNA as a minor profile across all other samples (7/8). Determination of the minimum number of contributors to these latter profiles showed DNA from at least two individuals in three samples, and from at least three individuals in five samples (Table 3). For the samples that showed at least two contributors, comparison of the DNA profiles obtained from these samples (underarm cut-out, cuff mini-tape and pocket mini-tape in month 1) to the wearer reference profiles showed that they also contained DNA from non-wearer sources in addition to both wearers (Fig. 5(a)). These findings were generally replicated in month 2 (Fig. 5(b)), with DNA from the regular wearer observed as the major profile across all samples that produced interpretable profiles (7/7).

When these two wearers were reversed, with V3 the regular wearer and V1 the second wearer, the DNA proportions were also reversed for most samples across both months (Fig. 5(c) and (d)). For 11 of 15 amplifiable samples, DNA from the second wearer was the major profile with DNA from the regular wearer as a minor profile (Table 2). Exceptions to this were observed when DNA from the regular wearer and second wearer were in approximately similar proportions in 3/15 samples, and one sample showed the regular wearer DNA as the major profile with the second wearer DNA as the minor profile (Fig. 5(c) and (d)).

Although no obvious trends in DNA profile composition were observed across different sampling methods and locations for most of the hooded jumpers (Fig. 5), an exception was observed for the V2:V4 pairing. For the hooded jumpers regularly worn by V2 and then worn by V4 for four consecutive hours, the regular wearer's DNA was observed as the major profile in all cut-out samples (8/8), whilst the second wearer's DNA was observed as the major profile in most (7/8) of the mini-tape samples (Fig. 5(e) and (f)). Whilst this finding was reproducible for three of the four locations sampled across the two experiments, it was not observed in any of the other pairings of wearers (Fig. 5).

Of the 14 amplifiable DNA samples from the hooded jumpers regularly worn by V4 and then worn by V2, 13 samples across both months showed DNA from the regular wearer as the major profile with the second wearer as the minor profile (Fig. 5(g) and (h)). Comparison to the reference profile from V4′s romantic partner identified that the majority of the non-wearer DNA in these same samples was from the regular wearer's partner that persisted even after the hooded jumper was worn by the second wearer (Fig. 5(g) and (h)). The remaining sample was the mini-tape from the cuff in month 2, which gave a single-source profile of the second wearer (Fig. 5(h)) from a notably large amount of DNA (150 ng; Supplementary Table S1).

When the minimum number of contributors to each DNA profile obtained for the hooded jumpers worn by two wearers was considered, differences were observed depending on the manner by which the number was determined (Table 3). Of the 60 DNA profiles that were successfully generated, 23 showed that the minimum number of

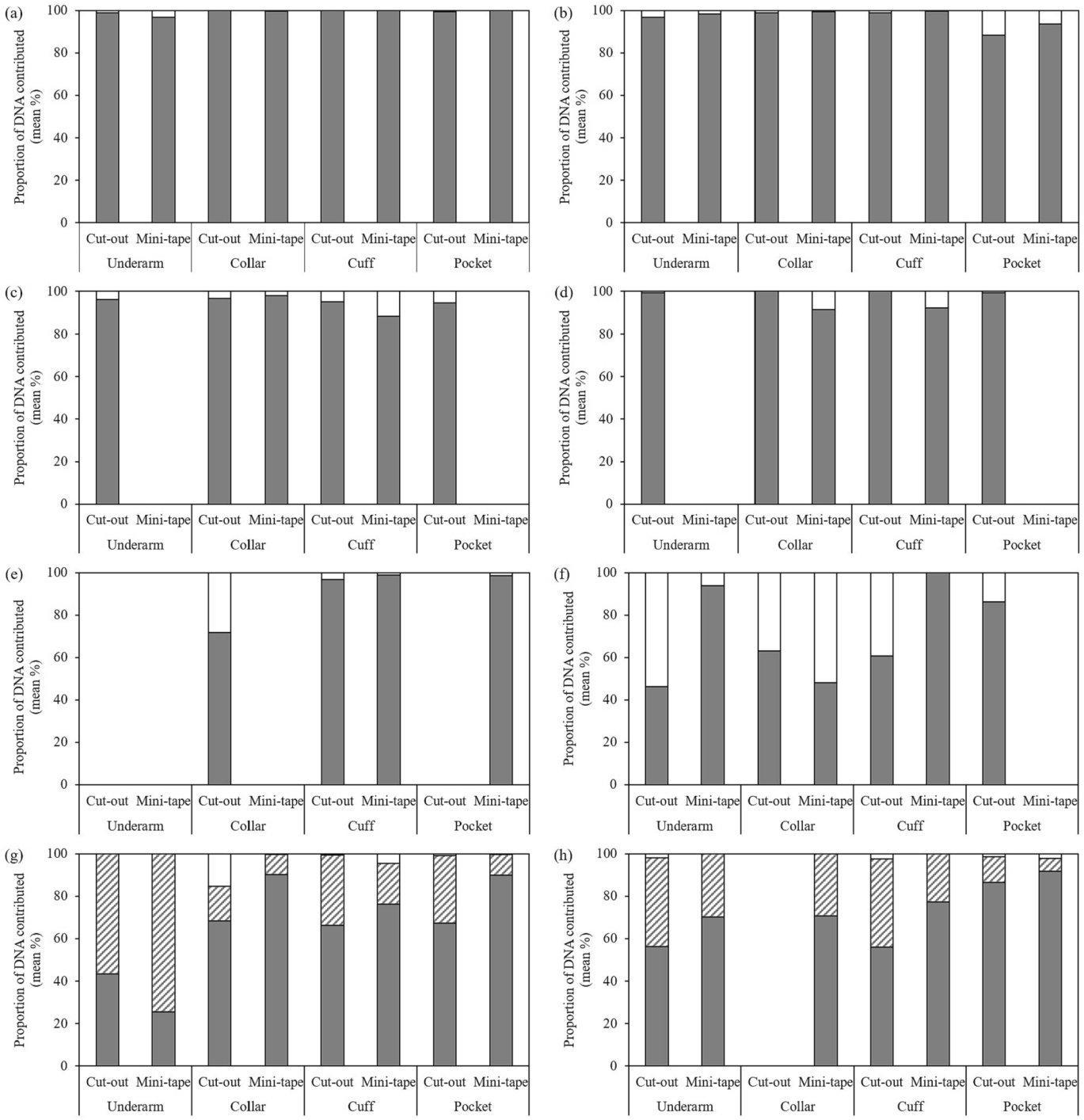

Fig. 3. Proportions of DNA contributed to DNA profiles generated from samples taken from regularly-worn hooded jumpers in months 1 and 2 by V1 (a) and (b), V2 (c) and (d), V3 (e) and (f), and V4 (g) and (h). Comparison to reference DNA profiles identified DNA originating from the regular wearer (grey), non-wearer sources (white) and the romantic partner of V4 (diagonal lines).

contributors was lower when interpreting the profiles without comparison to reference profiles than when reference profiles of the participants and V4′s romantic partner were considered (Table 3).

4. Discussion

This study is the first to investigate the composition of DNA profiles recovered from items of clothing, as opposed to accessories such as bracelets, sweat bands and gloves [3,17,18,20,21], that have been authentically regularly worn, with only the duration of wear and frequency of washing controlled, and then been worn by a second wearer. Whilst over 90 % of the DNA profiles obtained from these hooded jumpers gave mixtures with a major profile, the major profile varied as to whether it could be attributed to the regular or the second wearer. Building on preliminary data presented by Harris *et al.* [16] and Dziak *et al.* [3], this study systematically examined whether using a different recovery method or sampling location might distinguish between DNA deposited by regular and most recent wearers of clothing, but no differences in DNA amount or profile composition, which were generally reproducible across wearer pairings and both replicates of the

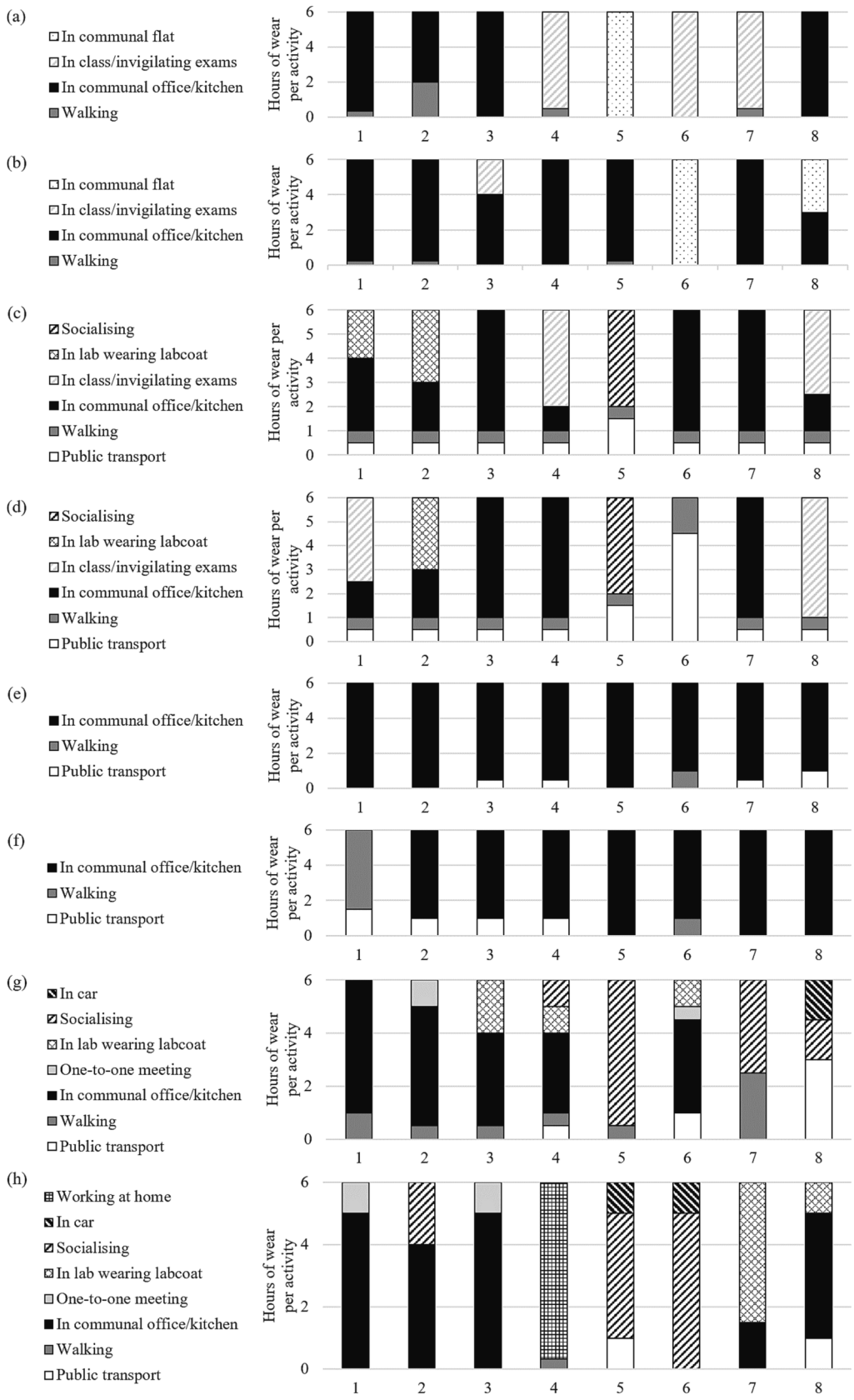

Fig. 4. Activities of participant during regular wearing of hooded jumpers. V1 month 1 (a) and month 2 (b); V2 month 1 (c) and month 2 (d); V3 month 1 (e) and month 2 (f); V4 month 1 (g) and month 2 (h). Hooded jumpers were laundered by each participant (Table 1) between days 2 and 3, days 4 and 5, and days 6 and 7. N. B. Whilst volunteers were asked to wear their jumpers for six hours per day, V3 accidently wore their jumper for 10 h on day 4 in month 2.

Table 2
Composition of DNA profiles recovered from the hooded jumpers worn by a regular wearer and then by a second wearer for four consecutive hours. For the purpose of making general observations, DNA attributed to V4′s romantic partner on V4′s jumpers is categorised here with the other non-wearer DNA detected; more detail of the profile compositions for all samples is shown in Fig. 5. M1, month 1; M2, month 2.

			V1:V3		V3:V1		V2:V4		V4:V2		Total: M1	Total: M2	Total: Overall
Single-source	Major component	Minor component	M1	M2	M1	M2	M1	M2	M1	M2			
Regular	–	–	1	0	0	0	0	0	0	0	1	0	1
Second	–	–	0	0	0	0	0	0	0	1	0	1	1
–	Regular	Second	2	0	0	0	0	2	0	0	2	2	4
–	Regular	Second, Non-wearer	5	7	1	0	4	3	7	6	17	16	33
–	Second	Regular	0	0	1	0	0	0	0	0	1	0	1
–	Second	Regular, Non-wearer	0	0	5	5	4	3	0	0	9	8	17
–	Regular, Second	Non-wearer	0	0	1	0	0	0	0	0	1	0	1
–	Regular, Second, Non-wearer	–	0	0	0	2	0	0	0	0	0	2	2
		Total	8	7	8	7	8	8	7	7	31	29	60

experiment, were observed among recovery methods or sampling locations.

The majority of samples taken from hooded jumpers that were worn by one or two individuals yielded quantities of DNA that ranged from 0 to ~ 40 ng, with an outlier of ~ 150 ng also observed. Such a wide range of DNA quantities has previously been observed for worn upper garments in a study by Szkuta *et al.* 2019 [8], in which they reported that most DNA quantities recovered also fell in the 0–40 ng range. They also observed samples, including internal and external surfaces of the garments, that contained more than 100 ng DNA. Therefore, a large degree of variation in the quantities of DNA recovered appears to be an expectation for worn items of clothing. In addition, observing tens, or even hundreds, of nanograms of DNA in samples from regularly-worn clothing provides further evidence that caution should be taken if using the quantity of DNA recovered from an item in casework to infer that the DNA came from a particular body fluid [24].

With respect to the effects of number of wearers (one or two), recovery method used (cut-out or mini-tape) and sampling location (cuff, collar, underarm or pocket) on DNA amounts recovered, no reproducible trends across all pairings of volunteers and both replicates of the experiment were observed. Given the expected accumulation of DNA on the jumpers through regular use, it is unsurprising that the additional wearing of the jumpers for just four hours by another individual did not reproducibly affect the amount of DNA recovered. The observation of no reproducible effects of using different recovery methods and sampling locations in this systematic experiment of these variables confirms the preliminary conclusions of Harris *et al.* [16] and Dziak *et al.* [3], that varying the sampling location or method for DNA recovery from clothing does not significantly affect the DNA amounts recovered. Whilst it is expected that cut-outs would generally recover more DNA than taping, cut-outs are limited to the size that will fit into a tube for extraction, whereas taping can be used on a larger surface area. In this study, the cut-outs used were approximately 1.5 cm x 0.5 cm, whereas the taped surfaces areas were larger (Section 2.2), which may explain why similar amounts were seen with both these methods. Since cuffs and collars are routinely sampled in casework, and similar sized areas were sampled for both locations, it was expected that these two areas would yield similar quantities of DNA, which is what was observed. Given the level of contact with skin and sweat that the underarm area has with a wearer, it is also not surprising that the DNA quantities recovered were similar to those recovered from the collars and cuffs, even though the size of the area taped was smaller. It was however surprising to see similar DNA amounts from the inside pockets, as the level of direct contact between a wearer's skin and the pockets would depend on how often they put their hands into the pockets. This could have been counteracted by redistribution of DNA between surfaces on the jumper during the laundering process [25], which may explain why similar amounts were recovered from the inside pockets as the other areas sampled.

However, reproducible differences in DNA deposition on worn hooded jumpers were seen between different wearers, with V4 consistently depositing significantly more DNA than V2 and V3 in both replicates of the experiment. This demonstrates that the concept of 'shedder status', when different individuals deposit different amounts of DNA, is not just applicable to items that are handled, but also applies to items that are worn, thereby supporting Szkuta *et al.*'s [8] hypothesis that some individuals deposit DNA to their clothing more readily than others and Otten *et al.*'s [19] observation of this for worn gloves. As discussed by Szkuta *et al.* [8], this does not just refer to DNA directly from the wearer being deposited on the clothing, but also includes the ability of an individual to collect DNA from other sources onto their clothing. This is important to note when considering V4′s jumpers. Significantly more DNA was recovered from V4′s jumpers than the other volunteers' jumpers in month 1 and than V2 and V3′s jumpers in month 2 (Fig. 2). This higher level of DNA deposited on V4′s jumpers was likely exaggerated by the presence of DNA from their romantic partner, which was observed in all amplifiable samples from their jumpers (Fig. 3(g) and (h)). Calculation of effect size showed a large effect of shedder status in month 1 with 71 % of the variability for both V2 and V3 being accounted for by the difference in individual wearing the hooded jumpers. However, this was reduced to a medium effect in month 2, only accounting for ~ 30 % (36 % for V2 and 30 % for V3) of the variability. This demonstrates that whilst shedder status plays an important part in affecting DNA deposition between wearers, the degree of that effect can vary. In this experiment, steps were taken to try to minimise variation between the two replicates of the experiment as much as possible, for example, through using hooded jumpers of the same material and maintaining the same durations of wear. However, this change in effect size between months suggests another variable (or variables) had an increased impact on DNA deposition in month 2 than month 1. This is also supported by the finding that V1 deposited significantly more DNA in month 2 than month 1, with 40 % of the variability being accounted for by the difference in month. One possible such variable is temperature, as the replicate of the experiment was conducted in June, which was a hotter month than May when the experiment was first run.

When the DNA profiles recovered from the hooded jumpers worn regularly by one wearer are considered, there is much less variability in the composition of the profiles than in the aforementioned DNA quantities. Whilst a few samples showed single-source profiles attributed to the wearer (9.1–18.8 %) for hooded jumpers worn by V1-V3, the majority (72.7–83.3 %) of the samples for all wearers across both months comprised a major profile of the wearer with a minor profile of non-wearer alleles, demonstrating a recurring observation of the presence of non-wearer DNA. This is consistent with the findings of other studies on clothing worn by only one person (e.g. [8,26]), for which it is most common to observe mixtures of DNA with a major profile attributable to the wearer. Although the three wearers V1-V3 lived alone, they all used a communal washing machine in their respective apartment buildings (Table 1) and spent much of their time wearing the jumpers engaged in activities that involved contact with surfaces potentially containing

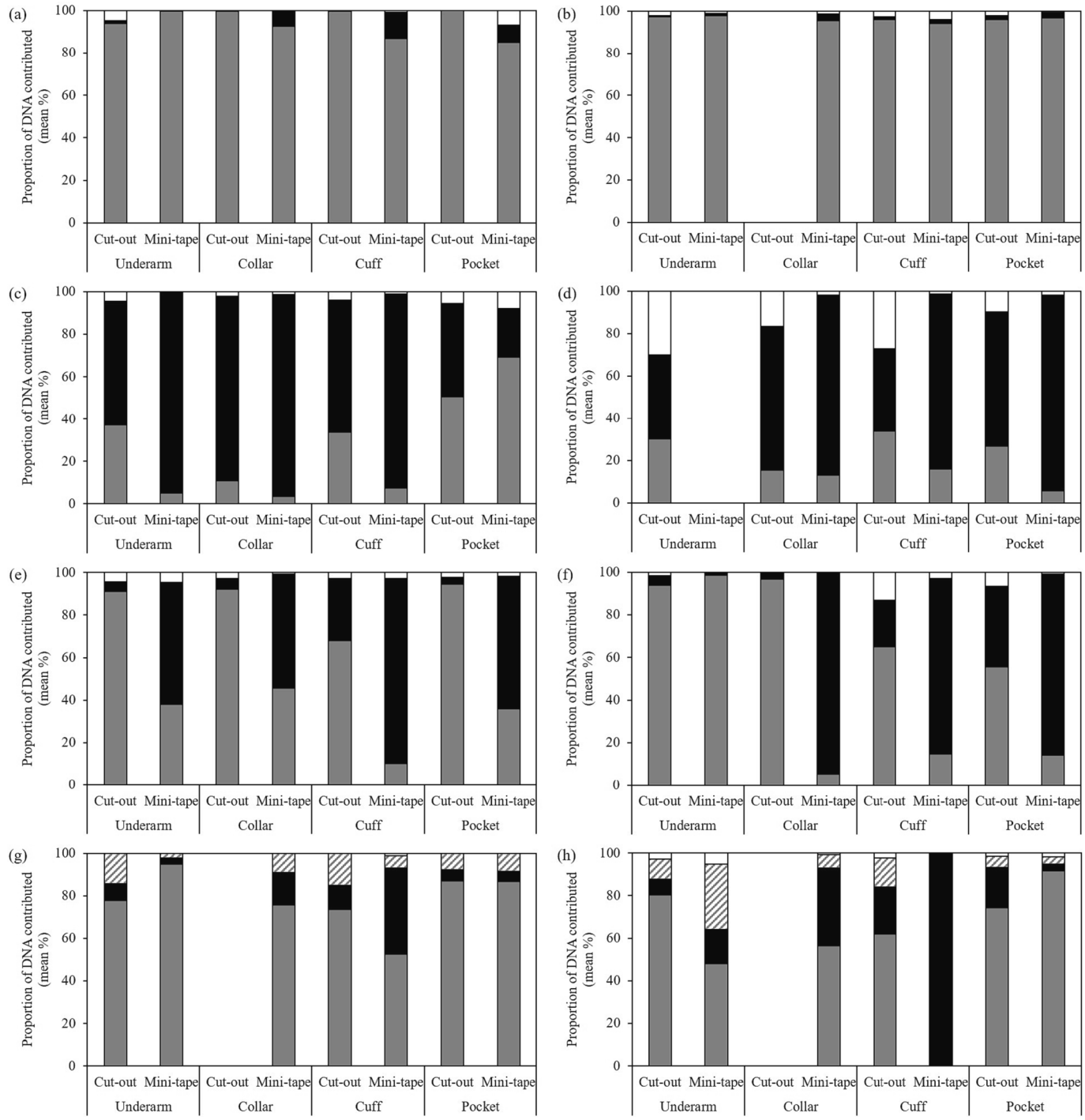

Fig. 5. Proportions of DNA contributed to DNA profiles generated from samples taken from regularly-worn hooded jumpers after being worn by a different wearer for four consecutive hours in months 1 and 2 by V1:V3 (a) and (b), V3:V1 (c) and (d), V2:V4 (e) and (f), and V4:V2 (g) and (h). Comparison to reference DNA profiles identified DNA originating from the regular wearer (grey), second wearer (black), non-wearer sources (white) and the romantic partner of V4 (diagonal lines).

other people's DNA (e.g. use of public transport, in communal office/kitchen, invigilating student exams etc; Fig. 4), which could explain the presence of non-wearer DNA. For V3, 18.2 % of samples also showed approximately equal proportions of wearer to non-wearer DNA, and many samples from V3's jumpers showed higher proportions of non-wearer DNA than for samples from jumpers worn by V1 and V2 (Fig. 3), even though their aforementioned circumstances were similar. Although V3 sometimes washed and stored their clothes, including their hooded jumpers, with one other individual (Table 1), this could not explain this finding, as the non-wearer component to their profiles tended to be complex mixtures rather than a recurring single profile.

For hooded jumpers only worn by V4, 20.0 % of their samples showed approximately equal proportions of wearer to non-wearer DNA and much of the non-wearer DNA on all their samples could be attributed to their romantic partner, including one sample in which DNA from the romantic partner was the major profile (Fig. 3 (g) and (h)). As V4 lived and shared a washing machine with their romantic partner (Table 1), the finding of their DNA is not unexpected, given previously published observations of DNA on worn clothing from close associates, including romantic partners, co-habitants and family members [7,8,16].

Table 3
Numbers of contributors to the hooded jumpers worn by a regular wearer and then by a second wearer for four consecutive hours. Numbers without parentheses show the minimum number of contributors based on allele count and relative peak height; numbers within parentheses show the minimum number of contributors based on comparison to the reference DNA profiles illustrated in Fig. 5; and underlined numbers indicate a discrepancy in the number of contributors between these two methods. Numbers are absent from those samples that did not generate an informative DNA profile, as per Supplementary Table S1.

Regular:Second wearer	Month	Underarm		Collar		Cuff		Pocket	
		Cut-out	Mini-tape	Cut-out	Mini-tape	Cut-out	Mini-tape	Cut-out	Mini-tape
V1:V3	1	2 (3)	2 (2)	2 (3)	2 (3)	2 (2)	2 (3)	1 (1)	2 (3)
	2	3 (3)	2 (3)	–	3 (3)	2 (3)	3 (3)	3 (3)	3 (3)
V3:V1	1	3 (3)	2 (2)	3 (3)	2 (3)	3 (3)	2 (3)	3 (3)	3 (3)
	2	3 (3)	–	3 (3)	3 (3)	3 (3)	3 (3)	3 (3)	2 (3)
V2:V4	1	3 (3)	3 (3)	3 (3)	3 (3)	3 (3)	3 (3)	2 (3)	3 (3)
	2	2 (3)	2 (2)	2 (2)	2 (3)	3 (3)	3 (3)	3 (3)	3 (3)
V4:V2	1	3 (3)	2 (3)	–	3 (4)	3 (3)	3 (4)	3 (3)	2 (3)
	2	3 (4)	3 (4)	–	3 (4)	3 (4)	1 (1)	3 (4)	2 (4)

However, the observation of their partner's DNA as a co-major or major profile in a fifth of the samples from V4′s regularly-worn hooded jumpers is surprising, especially when it is common in casework to infer that the major profile comes from a wearer of the item (as discussed in the Introduction). In addition, the lack of single source DNA profiles from V4 on their hooded jumpers is likely explained by the prevalence of DNA from their partner.

For the two pairings of V1 and V3, opposing results were observed; DNA from the regular wearer was the single-source or major DNA profile in all samples from V1′s jumpers, whereas DNA from the second wearer was the major or co-major DNA profile in all but one of the samples from V3′s jumpers (Fig. 3). Although no significant differences were observed between the total amounts of DNA initially deposited on the hoodies worn by each of these two volunteers (Fig. 2), it was observed that substantially more DNA from non-wearer sources was present on V3′s jumpers than those of V1 (Fig. 3). As such, V3 appeared to deposit less of their own DNA onto their jumpers prior to V1 wearing the jumpers, therefore resulting in the DNA from the second wearer V1 being more dominant. This poorer shedding ability of V3 also explains the lower proportion of V3′s DNA as the second wearer on V1′s jumpers, such that the DNA of the regular wearer V1 remained dominant.

When V2′s jumpers were worn by V4, this was the only pairing that saw an observable difference in DNA recovered between each recovery method, since the mini-tape samples tended to show a major profile of the second wearer, whereas the cut-out samples tended to show a major profile of the regular wearer. Whilst these findings were reproducible across both replicates of the experiment for the collar and cuff, locations that are routinely examined in casework, these findings were only observed for this pairing. It is unclear why this was the case. When the pairing was reversed, all but one of the samples from V4′s jumpers that had been worn by V2 showed a major profile from V4, the regular wearer. Given that V4 deposited significantly more DNA than V2 (Fig. 2), it is not surprising that their DNA persisted as the major profile even after V2 had worn the jumpers; a similar finding has been observed for multiple users of items when the regular user is a significantly better DNA shedder than the second user [13]. The exception to this was the mini-tape of the cuff in month 2, which showed a single source DNA profile of the second wearer. As 150 ng of DNA was detected in this sample, far more than all the other samples, this suggests that the cuff may have been used by the second wearer for a 'DNA-loading activity', such as wiping their nose or mouth, thereby transferring additional sources of DNA, such as nasal mucous or saliva. Interestingly, even after V4′s jumpers had been worn by V2, DNA from V4′s romantic partner still persisted on the jumpers in all but the aforementioned single-source sample (Fig. 5(g) and (h)).

Overall, the results obtained for each pairing of wearers were generally reproducible across both months of the experiment, providing support for the idea that having volunteers perform a more realistic casework-relevant activity once for the purposes of generating data to inform activity level probabilities [24] is sufficient to have confidence in the dataset. Differences in the profile compositions were instead observed between pairings, supporting the substantial impact of shedder status on the results obtained, as discussed earlier. Taken together, these two observations indicate that this experiment should be repeated with a higher number of different pairings to accommodate the variations in DNA deposition between wearers in order to generate the data required for activity level evaluation in casework regarding multiple wearers. However, this experiment itself provides preliminary data for this purpose showing that ~ 60 % of profiles obtained gave a major profile of the regular wearer, whereas ~ 30 % gave a major profile of the second wearer; these findings being reproducible across both months of the experiment (Table 2). The remaining profiles comprised other much less frequent observations of single-source profiles of each wearer and equal proportions of DNA from both wearers. Repeating this experiment with an increased number of different pairings will provide confidence in the frequencies of these observations, particularly for those observed rarely, such as the major profile being from a non-wearer (romantic partner here), and those observed so rarely as not to have been detected in the four volunteer pairings used here.

The observation of reproducible STR profile contributions for each pairing also provides support for the suggestion that the relative proportions within mixed DNA profiles may be more informative than DNA quantity alone for activity level evaluations (as discussed in [24]). This can be seen when considering the relative proportions of different sources of DNA in this study (regular wearer, second wearer, romantic partner and non-wearer DNA) in relation to the varied DNA quantities recovered from the jumpers (Supplementary Table S1). For example, take the collar mini-tape from the hoodie worn by V1 and then by V3 in month 1. The proportion of DNA attributed to the regular wearer for this profile is 92.5 % (Fig. 5(a)), which equates to 1.9 ng, when multiplied by the total DNA (2.10 ng) recovered in this sample (Supplementary Table S1). Now take the collar mini-tape from the hoodie worn by V4 and then by V2 in month 1. The proportion of DNA attributed to the second wearer for this profile is 15.2 % (Fig. 5(g)), equating to 1.2 ng, when multiplied by the total DNA recovered (8.00 ng) in this sample (Supplementary Table S1). This example illustrates that, while the proportions of DNA from different sources (regular versus second wearer) differed between the two jumpers, the amounts attributed to the different sources were of the same magnitude.

Finally, it is important to note that interpreting whether DNA could be attributed to each wearer was done herein by comparing the profiles obtained with the reference profiles from the wearers and V4′s romantic partner. As such, alleles from the second wearer were observed in all but one of the samples from all the jumpers worn by two wearers. On the surface, this supports Poetsch *et al.*'s [21] observation that DNA from the second wearer could be recovered from all sweatbands worn by the second wearer for four hours, the same duration as in this experiment. However, reference profiles are not always available in casework, and as such, profile interpretation will often start with determining the minimum number of contributors. Over half of the interpretable profiles

obtained from the jumpers worn by two wearers in this experiment were interpreted as having fewer contributors in the absence of reference profiles than with comparison to reference profiles (Table 3). This means that alleles from the second wearer were only identified because of comparison of the sample profiles to the reference profiles. As such, if the minimum number of contributors was determined prior to interpretation, these profiles would have been interpreted as having fewer contributors than they actually had, such as being interpreted as two-person mixtures of a major and minor profile, rather than three-person mixtures of a major and two minor profiles (Table 3). When the wearer reference profiles were then compared, the single minor profile would not have corresponded with a reference profile and the second wearer would likely be excluded as a contributor, especially given the low proportions of second wearer DNA in many of the profiles obtained (Fig. 3). Whilst this requires further investigation, probabilistic genotyping methods are being evolved to overcome the need to assign the number of contributors, which may negate this potential issue.

Overall, our results reinforce existing data in the literature on the need for caution when interpreting DNA profiles obtained from clothing. One should avoid hasty conclusions about the origin of DNA, highlighting not only the possibility of using an item by more than one person, but also addressing the possibility of DNA transfer (e.g. in shared washing machines). Information regarding these possibilities should be considered when evaluating DNA traces given activity level propositions, such that likelihoods of observing the DNA profiles given different propositions can be appropriately determined.

CRediT authorship contribution statement

Georgina E. Meakin: Conceptualization, Methodology, Funding acquisition, Investigation, Visualization, Formal analysis, Writing – original draft, Writing – review & editing. **Guilherme S. Jacques:** Methodology, Investigation, Visualization, Writing – review & editing. **Ruth M. Morgan:** Conceptualization, Methodology, Funding acquisition, Supervision, Writing – review & editing.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scijus.2024.02.001.

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