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Contacting surfaces are rarely DNA Free: Another look at transfer when both surfaces have DNA

Georga Sallows [a], Duncan Taylor [a,b], Roland A.H. van Oorschot [c,d], Mariya Goray [a,*]

[a] Flinders University, Adelaide, South Australia 5042, Australia
[b] Forensic Science South Australia, Adelaide, South Australia 5000, Australia
[c] Office of the Chief Forensic Scientist, Victoria Police Forensic Services Department, Macleod, Victoria 3085, Australia
[d] School of Agriculture, Biomedicine and Environment, La Trobe University, Bundoora, Victoria 3086, Australia

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ABSTRACT

Understanding DNA transfer, persistence, prevalence, and recovery (TPPR) has become increasingly crucial in forensic investigations. Most DNA transfer studies have focused on one-way transfer, neglecting bi-directional exchange. This study examines two-way transfer of blood and touch DNA between plastic and cotton substrates under passive and friction conditions, using methods from previous unidirectional transfer experiments.

Our investigation established statistically significant correlations between bi-directional DNA transfer rates and manner of contact but not substrate type and type of biological material. While, in general, no significant differences were noted between bi-directional and unidirectional transfer rates, significant differences were observed for certain variable combinations where unidirectional transfer resulted in higher transfer rates. This research provides baseline data on bi-directional DNA transfer under semi-controlled conditions, complementing existing unidirectional transfer knowledge. Understanding bi-directional transfer is crucial for accurately modelling DNA transfer events in forensic scenarios, especially for activity level evaluations.

1. Introduction

The discovery that DNA can be detected from non-visible biological materials, left behind by touching a surface with a hand, has transformed the field of forensic biology [1]. The enhanced sensitivity of touch and trace DNA analysis, while groundbreaking, has introduced questions regarding transfer mechanisms and deposition pathways in courtroom deliberations [2,3].

DNA transfer can occur unidirectionally, where genetic material moves from one surface or donor to another, and bi-directionally, where an exchange of DNA takes place between two surfaces during a single interaction [2]. Research has demonstrated that the rate of unidirectional DNA transfer is influenced by multiple factors, including the nature of the substrate [4–7], the manner of contact [4,5,8], the moisture content of the sample [4,5] and the type of the biological material involved [4,5,9]. Yet the effects of these variables during bi-directional transfer remain unknown.

The relevance of bi-directional transfer is obvious. Background DNA (bDNA) is a ubiquitous feature of crime scenes, present on most public and private items [1,10–16]. It is, therefore, reasonable to expect that when two surfaces come into contact in a crime scene context, bi-directional transfer occurs. For example, when a person of interest contacts a surface at the scene of the crime, both the individual in question and the contacted item are likely to have DNA on them, and the exchange of this biological material ensues in a bi-directional manner. Taylor et al. [17,18] emphasize the critical importance of incorporating bDNA when constructing Bayesian Networks for forensic analysis. Neglecting bDNA can lead to unrealistic shifts in proposition support. Furthermore, this consideration should be extended to include transfer rates between objects that both contain DNA deposits.

Taylor et al (see section 4.2 of [19]) highlight the need for data on bi-directional transfer and note the lack of currently available information: "It is not clear from literature whether the transfer in both directions should be tied together i.e., if 10 % of DNA transferred from site 1 to site 2 during a contact do we also expect 10 % of DNA from site 2 to transfer to site 1? And how does differing starting DNA amounts on site 1 and site 2 affect this expectation?". The present study adapts the methods developed by Goray et al [4,5] to investigate and compare bi-directional transfer of DNA with unidirectional transfer across various combinations of substrates (cotton and plastic), contact types (passive and friction),

* Corresponding author at: College of Science and Engineering, Flinders University, Adelaide, South Australia 5042, Australia.
E-mail address: mariya.goray@flinders.edu.au (M. Goray).

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and biological materials (blood and touch).

2. Materials and methods

2.1. Biological material and substrate type

For touch DNA, to maximise the quantity of DNA available for deposits, participants rubbed their fingertips along the nape of their neck (just below the hairline) 15 min after washing hands. A primary deposit of touch DNA was established by rubbing one of the following six fingers: the thumb, pointer or index finger of the right or left hands, onto one of the six deposit areas for 10 s. This created six deposits in a single session. Two participants (male and female) were used for bi-directional touch transfer and experiments were conducted within 15–30 min of deposit. The female donor (called primary donor or depositor in the paper) was always placed as bottom substrate and male donor (called secondary donor or depositor in this paper) was always the top substrate. For blood deposits, 15 μL of blood from the same female participant (collected into a tube containing EDTA to prevent coagulation before use) was deposited in the centre of the deposit square and dried at room temperature for 18–20 h prior to transfer experiments. The male participant served as the touch DNA donor for touch/blood bi-directional transfer experiments. The deposits were made either on cotton (100 % precut calico natural, Spotlight) or plastic transparency (Plain Paper Copier Transparency Film, Nobo); also used as two intermediary substrates and stencil). Each side of cotton fabric was exposed to UV light for 30 min while both sides of plastic were cleaned with1% hypochlorite, distilled water and 70 % ethanol.

2.2. Experimental set up

The study adapted and modified the methodologies outlined by Goray et al [4,5]. The transparency stencil (8 cm x 10 cm) with a 1 cm x 1 cm deposit square (and a 1 cm boarder around the deposit square) was overlayed with two sterilised transparencies (to prevent contamination) followed by the deposit substrate (cotton or transparency plastic) and secured together at the sides with clean sticky tape (Fig. 1).

The touch or blood deposit was made inside the deposit square outlined by the stencil. One substrate with a deposit of biological material was then placed on top of another substrate also containing a deposit of biological material in such a way that the two deposit areas were aligned and in contact with each other. Two contact types were tested (see section 2.3).

2.3. Contact type

Two types of contact were applied: ‘passive’ and ‘pressure with friction’ (subsequently referred to as ‘friction’). Under passive contact, the two substrates were placed on top of each other with the deposit substrates aligned and in contact for 1 min. Under friction contact, conditions identical to passive contact were applied, except the top substrate (always containing male participant’s deposits) was secured to a 1 kg weight that was moved in all directions during the contact (Fig. 2). The substrate was affixed to the weight with sticky tape. Furthermore, during friction contact, movement of the weight was restricted to the outer deposit boarder (1 cm). The weight was moved by hand in all four directions, but not extending past the 1 cm boarder that was stencilled around the deposit. The movement involved moving the weight in one direction followed by return to the original deposit location then to the next direction until four movements were completed (i.e. left, right, up and down). The process was then repeated for 1 min. The surrounding area outside the original deposit square was co-extracted to capture any biological material spread outside the initial deposit square.

The touch (male)/touch (female) and touch (male)/blood (female) bi-directional transfers were examined with all combinations of tested substrates (cotton/cotton, cotton/plastic, plastic/cotton and plastic/plastic) and contact types (passive and friction) in four replicates resulting in 128 samples for DNA analyses.

2.4. Sample processing and control samples

The substrate deposit area and adjacent underlying transparency (1 cm x 1 cm) including surrounding margin (1 cm) were excised and processed together. DNA was extracted with DNA IQ™ in a final volume of 60 μl (Promega®), quantified with Quantifiler™ Trio DNA (Applied Biosystems™), amplified with the PowerPlex®21 multiplex kit (Promega®; 30 cycles) and typed with 3500xL Genetic Analyser (24 s, 1.2 kV) and GeneMapper™ IDx (v. 1.6) Applied Biosystems™; 175RFU detection threshold). The DNA amount used in amplification was 0.5 ng or 15 μl if the concentration was $\leq$ 0.033 ng/μl. All samples were progressed to amplification, irrespective of the quantification result.

Three control samples, obtained from cleaned cotton and plastic substrates as well as the work surface, all yielded negative results during both quantification and DNA typing.

2.5. Data analysis

The total DNA amount (ng) on each of the two substrates after the tested contact was calculated by multiplying sample DNA concentration (ng/μL) by the elution volume (60 μL). When samples were taken (after the contact), the DNA profiles from each substrate could contain DNA from the primary donor, DNA that has been secondarily transferred, and unknown DNA. It is not possible to determine where the unknown DNA has originated in all situations, so the percentage and amount of DNA transferred is determined from the known donors only. The starting amount of a donor was calculated as the sum of the DNA of that donor detected on both substrates.

The minimum number of contributors was manually determined utilising the maximum allele count (MAC) method in combination with peak height balance. STRmix™ v2.9 (Institute of Environmental Science and Research (ESR) and Forensic Science South Australia (FSSA)) was used to determine mixture proportions and person of interest inclusions/exclusions. For mixed DNA profiles STRmix™ mixture proportions were used to allocate amount contributions.

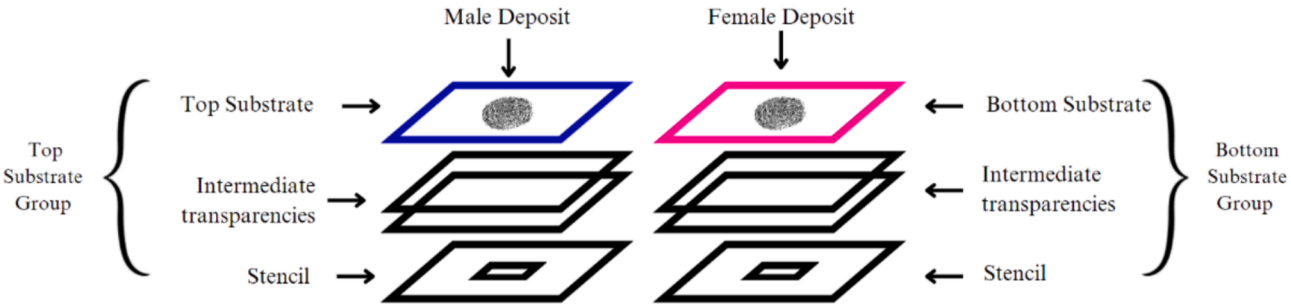

Fig. 1. Experimental set up for male (blue; touch DNA) and female (pink; touch DNA or blood deposits prior to contact.

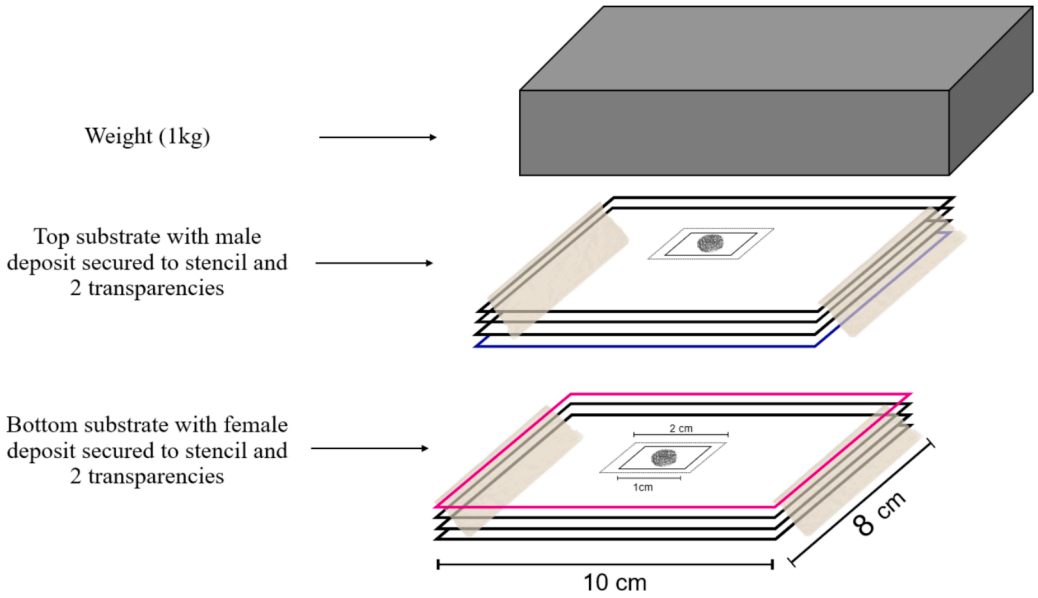

Fig. 2. Experimental set up for the friction experiment with female (bottom; pink) and male (top; blue) deposits (not to scale) with substrates affixed together and to the weight with sticky tape.

The percent (%) transfer of DNA was calculated as described by Goray et al [4]. Firstly, the DNA amounts (ng) were determined by multiplying the volume of the extract by its concentration. The total amount was then divided between all contributors based on the mixture proportions (for all included individuals based on the LRs) derived from STRmix™. The donor deposits detected on one or both surfaces (where applicable) were added together to determine the total amounts deposited and these donor specific amounts were used in the transfer calculations. The percent transfer was derived by dividing the total donor amount detected on the secondary substrate by the total donor DNA amounts. To assess potential differences between unidirectional and bi-directional DNA transfer, data generated in this project was compared with the data generated by Goray et al [4,5].

Multiple linear regression, linear regression, ANOVA and Kruskall-Wallis (K-W) tests were performed to determine if there were any statistically significant differences ($p < 0.05$) between variable groups using R Studio (v. 4.2.3) and IBM SPSS v. 29.0.1.0. Effect size, d, was calculated using the method of standardised effect size referred to as Cohen's d [20] using R package 'effectsize'. We follow the recommendations of Cohen for interpreting the effect size i.e. a standardized effect size:

$d < 0.2$ – Very small
$0.2 <= d < 0.5$ – Small
$0.5 <= d < 0.8$ – Medium
$d >= 0.8$ – Large

The choice of when an effect size is impactful to the practical application of a model to real-world observations is context dependant (i.e. how the data and model will be used) and largely a subjective choice based on domain knowledge. We subjectively choose to consider at least a medium effect size to be of practical importance in this context i.e. greater than 0.5.

3. Results

In the experiments, both cotton and plastic substrates served dual roles as primary and secondary substrates, each receiving initial deposits. For clarity, we defined the 'primary substrate' as the one bearing the initial deposit from the donor under assessment, while the 'secondary substrate' denoted the substrate to which this donor's biological material subsequently transferred. This reciprocal relationship means each substrate functions as both primary and secondary, depending on the perspective. Within the following sections, when discussing 'substrate-to-substrate' transfer, the primary substrate is consistently listed first.

3.1. Amounts of DNA deposited and proportions transferred

The average donor deposited amounts of DNA for each biological material and surface type are available in Table 1. The amount of DNA deposited onto primary substrates varied depending on biological material and substrate type (Supplementary material 1, tab 1). Blood deposits resulted in a significantly higher amount of DNA than touch deposits (Table 1; $p < 0.05$, $d = 1.81$). The touch DNA amount deposits retrieved from cotton were significantly higher than on plastic ($p < 0.05$, $d = 0.32$) although the effect size was only small and so may not have practical effect on interpretations. Comparatively, for blood, more DNA was retrieved from deposits onto plastic than cotton ($p < 0.05$, $d = 12.02$) and the effect size for this observation was very large. No significant differences ($p > 0.05$) were noted between the two touch DNA

Table 1
Average donor deposit amounts (excluding unknown (u/k), mixture proportions and number of contributors (SD) and average donor % transfer rates.

		Touch		Blood
		Touch to touch	Touch to blood	Blood to touch
Av. deposit amount (ng) of donor DNA (excluding u/k)		0.351(range: 0.002–1.686)	0.241 (range: 0.001–1.1)	9.572 (range: 1.884–19.602)
Av. deposit amount (ng) of donor DNA based on surface type	Cotton	0.579 (range: 0.042–1.687)	0.265 (range: 0.032–0.586)	15.581 (range: 1.884–5.605)
	Plastic	0.124 (range: 0.002–0.79)	0.216 (range: 0.001–1.1)	3.564 (range: 11.328–19.602)
Av. Number of contributors		2 (SD 0.7; range 1–4)	2.4 (SD 0.5; range 2–3)	1.4 (SD 0.6; range 1–2)
Av. donor's % proportion of total deposit		84.8 % (range 0–100 %)	85.9 % (range 42.1–100 %)	99.8 % (range 97.5–100 %)
Av. % transfer rate of donor DNA		10 % (range 0–100 %)	7 % (range 0–78 %)	0.16 % (range 0–2 %)

donors (mean of 0.360 ng and 0.290 ng for female and male participants, respectively).

The linear regression analysis indicted that there was no significant relationship between initial deposit amounts on the primary substrate and transfer amounts to the secondary substrate (Fig. 3). A multiple linear regression analysis was conducted to examine the factors influencing the amount of donor DNA transferred. The model included several predictor variables including starting donor DNA amount, substrate type (both from and to), contact type, biological material of the donor and of the receiving substrate, along with an interaction term between substrate types. This analysis highlights that contact type significantly influences donor DNA transfer amounts and an effect size of practical significance ($p < 0.05$, $d = 0.62$), while starting initial DNA amount, biological material and substrate type do not appear to have a significant impact.

When all other variables were kept constant, plastic as primary (vs. cotton as primary; mean 9 % vs 4 %, respectively) and cotton as secondary substrate (vs. plastic as secondary; mean 10 % vs.4 %, respectively) resulted in greatest DNA transfer, however, this was not significant.

3.2. Bi-directional transfer

3.2.1. Transfer of touch − touch deposits

The mean percent DNA transfer rates (and SD) for touch-to-touch deposits are shown in Table 2. Both substrate and contact type were shown to impact the amount of DNA transferred from primary to secondary substrates. Irrespective of contact type, when plastic was a primary substrate, it facilitated more transfer (mean of 14 %; max of 100 %) than cotton, (mean of 7 %; max of 62 %), however this was not significant. Comparatively, cotton as a secondary substrate significantly increased the transfer rates compared with plastic (mean of 16.6 %; max of 100 % and mean of 4 %; max of 60 % respectively) ($p < 0.05$, $d = 0.46$), This had a medium effect size but is on the edge of whether the size is of practical significance. Therefore, a transfer event between plastic as the primary substrate and cotton as the secondary substrate results in the highest percent transfer (mean of 21 %; max 100 %), and cotton as the primary substrate and plastic as the secondary results in the lowest percent transfer (mean of 0.2 %; max 1 %) ($p < 0.05$, $d = 0.14$). However, the effect size for the difference between these transfer types is very small indicating that there is likely to be little practical difference seen in the means compared to the amount of variance seen in the observed transfer proportions.

Table 2
Mean % Transfer of DNA (SD) between primary and secondary substrate combinations under passive and friction contact (touch to touch).

	Secondary Substrate			
	Plastic		Cotton	
Primary Substrate	Passive	Friction	Passive	Friction
Plastic	4.42 (7.24)	11.32 (20.84)	0.27 (0.75)	40.78 (38.15)
Cotton	0.20 (0.41)	0.65 (1.57)	0.56 (0.82)	24.70 (19.92)

To assess the findings further, the percentage of the donor DNA transferred was analysed using a beta regression. The model included several predictor variables including starting donor DNA amount, substrate type (both from and to), contact type, biological material of the donor and of the receiving substrate, along with an interaction term

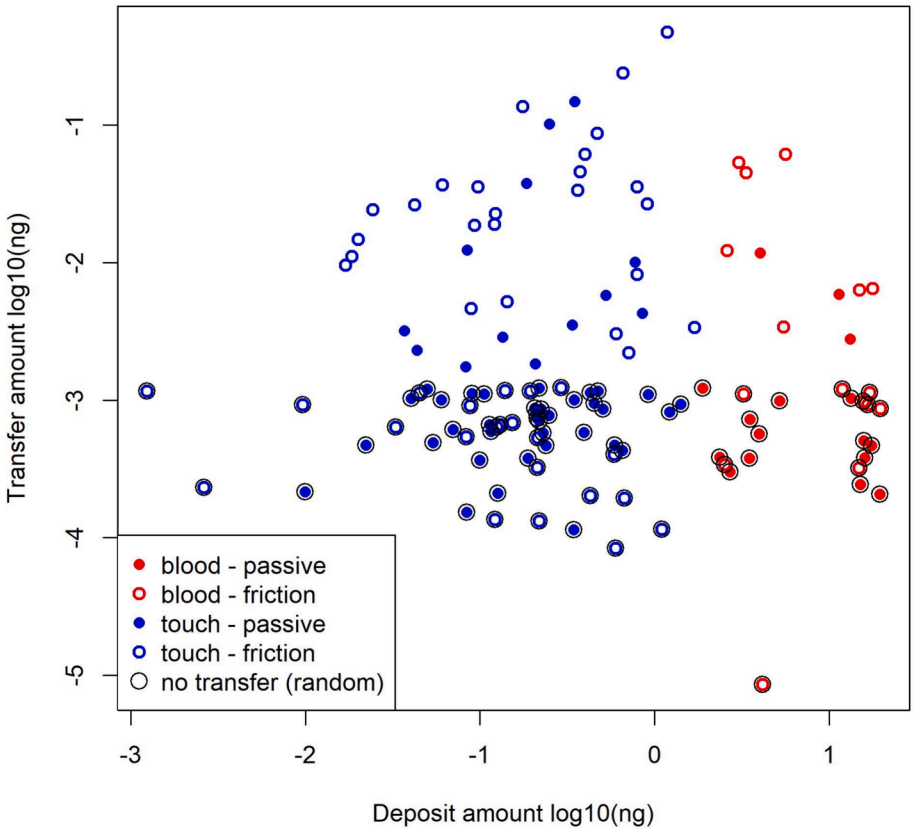

Fig. 3. DNA transfer amounts coming from a passive (closed circle) and friction (open circle) contact for blood (red) and touch (blue) deposits vs the deposit amounts. Points for which no transfer was observed were assigned a random value between 0 and the lowest observed DNA amount in the study (0.001 ng) and these points have been ringed by a black circle.

between substrate types. Again, the contact type significantly influences donor DNA transfer amounts ($p < 0.05$). Additionally, there were indications that the starting donor DNA amount and the substrate type may have an effect. On inspection of the model parameters the starting DNA amount coefficient was negative, indicating that the greater the starting amount, the lower the percentage transfer. The finding of no significant correlation between starting DNA and transferred DNA, whilst there being a significant impact of starting DNA on transfer percentage, could be explained by a relatively constant amount of DNA being transferred regardless of the starting amount. This mechanism seems counter intuitive and we suspect that the occurrence of many values of zero transfer is driving these findings.

Friction significantly increased transfer rates when secondary substrate was cotton (cotton to cotton $p < 0.001$; plastic to cotton $p < 0.01$), but not plastic (cotton to plastic $p = 0.61$; plastic to plastic $p = 0.52$).

3.2.2. Transfer of touch − blood deposits

The mean percent transfer and standard deviation of DNA transfer rates from touch to blood and from blood to touch deposits are shown in Table 3.

With touch deposits transferring to blood covered substrate, similar to touch-touch transfers, plastic primary substrate (mean of 9.4 %; max of 42.3 %) and cotton secondary substrate (mean of 10.0 %; max of 78.0 %) facilitated more transfer than cotton primary (mean of 5.80 %; max of 62.3 %) and plastic secondary substrates (mean of 5.2 %; max of 42.3 %) (Supplementary data 1). However, these differences were not significant. The highest touch transfer rate was observed from plastic to cotton under friction contact. Friction contact resulted in higher transfer rates for some substrate combinations (Table 3), however, it was not statistically significant ($p > 0.05$).

With blood deposits transferring to touch covered substrates, in contrast to touch deposits, cotton primary substrate facilitated more transfer than plastic, regardless of the secondary substrate (Table 3); however, linear regression showed no impact of substrate type on the DNA transfer percentages ($p > 0.05$). More variability was noted for secondary substrates. Under passive contact, higher transfer was to the plastic substrate, while under friction, higher transfer rate was to cotton secondary substrate. Comparatively to the transfer of touch DNA, a blood transfer event between cotton as both the primary and secondary substrate, under friction contact, resulted in the highest percent transfer. Friction contact resulted in an increase in transfer rates in all transfer events except plastic to plastic, however, overall, these were not statistically significant ($p > 0.05$).

3.3. Comparison of unidirectional and bi-directional transfer

The unidirectional data from Goray et al [4,5] (Supplementary data 1; tabs 2 and 3) was compared to bi-directional data, generated in this project, to assess the effects of substrate and contact type on transfer of DNA under these two conditions. For the bi-directional transfer, touch-touch (TT) results were combined as both substrates acted as primary substrates (Fig. 4).

Overall, transfer rates were lower under passive than friction contact and secondary substrate appears to play a role. In friction contact, cotton secondary substrate showed increased transfer compared to plastic. Conversely, under passive contact, more transfer was observed with plastic secondary substrates, however this was noted only in bi-directional transfer.

The data was also analysed to determine whether there was any indication that the DNA transfers occurring in one direction affected the transfer in the opposite direction i.e. if a higher percentage transferred from substrate 1 to substrate 2 (for a given biological material, substrate and contact type) was there any evidence that this resulted in a higher percentage transfer from substrate 2 to substrate 1. Fig. 5 shows the transfer percentage observed that occurred in both directions. Fig. 5 shows no obvious visual indication that the two transfers in a bi-directional contact are tied together, and statistical analysis confirmed the lack of significance.

3.3.1. Touch transfer

For touch deposits, unidirectional transfer [5] resulted in greater transfer rates than bi-directional transfer to touch DNA substrate (TT; mean of 13.4 % vs UD10.3 % respectively), however, this was not observed across all pairings (Table 4). For example, passive contact from plastic to plastic resulted in greater transfer during bi-directional transfer, but not significantly so.

In the comparisons of the unidirectional and bi-directional transfers, substrate had significant impact on transfer rates for certain combinations of variables. Under friction contact and transferring from cotton to plastic and under passive contact and transferring from plastic to cotton unidirectional transfer had significantly higher transfer rates than bi-directional transfer ($p < 0.05$; Table 4).

Similarly, bi-directional transfer of touch DNA to blood covered substrate was generally lower than unidirectional transfer (TB; mean of 13.4 % vs UD 7.59 % respectively), however, this was not observed across all combinations (Table 4). Significant differences were observed for plastic to plastic under friction contact where less was transferred during bi-directional transfer ($p < 0.05$).

Type of contact did not significantly impact transfer rates between unidirectional and bi-directional transfer.

3.3.2. Blood transfer

No statistically significant differences were noted for unidirectional [4] and bi-directional transfer of blood under passive or friction contact (Table 5).

In the comparisons of the unidirectional and bi-directional transfers, substrate had significant impact on transfer rates for certain combinations of variables. Under friction contact and transferring from plastic to both plastic and cotton significantly more blood transferred during unidirectional than bi-directional transfer ($p < 0.05$; Table 5).

Table 3
Mean % Transfer of DNA (SD) between primary and secondary substrate combinations under passive and friction contact (touch and blood).

	Secondary Substrate			
	Plastic		Cotton	
Primary Substrate	Passive	Friction	Passive	Friction
Touch to Blood:				
Plastic	10.6 (21.1)	0	3.66 (7.3)	23.41 (37.1)
Cotton	10.20 (20.4)	0	0	4.66 (9.36)
Blood to Touch:				
Plastic	0.02 (0.02)	0	0	0.02 (0.02)
Cotton	0.07 (0.15)	0.35 (0.66)	0	0.83 (0.77)

4. Discussion

The total amounts of DNA collected from substrates with touch deposits fell within the lower end of the reported ranges [21] and what was detected in the Goray et al [5], likely due to the smaller deposit size utilised in this study. Concordantly with Goray et al [5] significantly more touch DNA was deposited on cotton than plastic, likely from cells adhering among the fibres of the cotton material [5,7]. While the variability in the touch deposits between two substrates may be attributed to the propensity of touch DNA to transfer to smooth and rough substrates, blood deposit quantities were essentially standardised through the use of the same blood source, collection time and deposit volume. Yet, total amounts retrieved from plastic were significantly higher than cotton. Studies show that substrates can influence DNA extraction efficiency

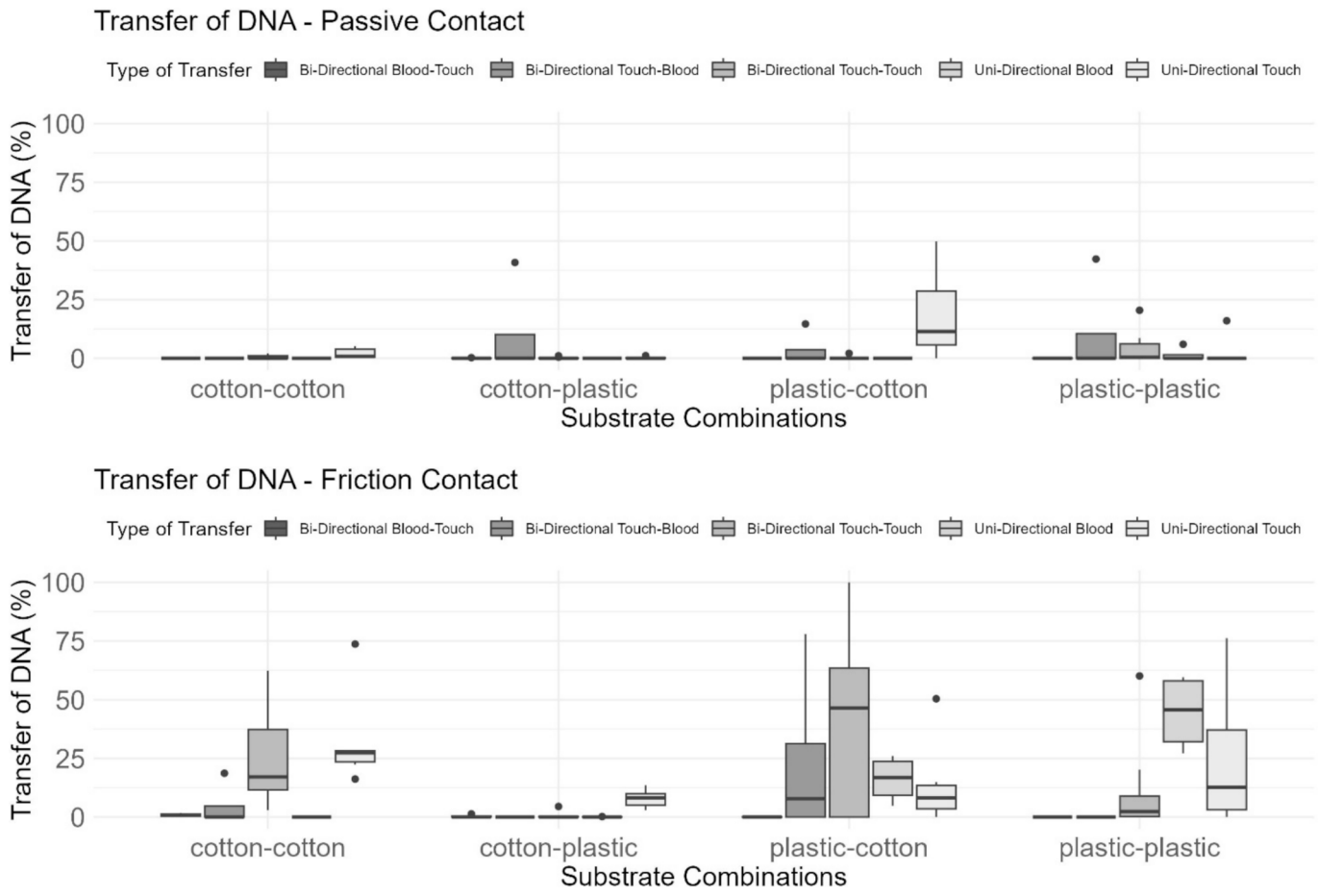

Fig. 4. Comparison of unidirectional and bi-directional transfer rates separate for each combination of substrates and passive and friction contact.

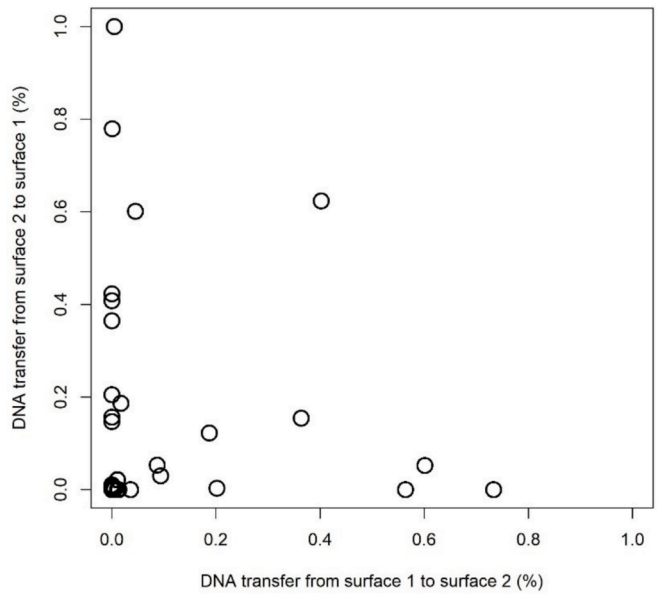

Fig. 5. DNA transfer (%) that occurred between substrates in a bi-directional contact experiment (across all combinations of substrate type, biological material and contact type).

[7,22] and future studies should explore further the effects of different substrates and how this may affect DNA transfer evaluations.

Previous research has shown that percent transfer remains consistent regardless of initial deposit amount [4,5] and this was confirmed in the present study where no significant relationship was noted between deposit and transfer amount. While percent transfer appears to be affected by the starting deposit amounts, we suspect that these differences are due to numerous zero values in the data.

Manner of contact impacted the transfer of DNA in all scenarios, with friction significantly increasing the transfer of touch biological material (touch-to-touch combinations). However, variability was noted for touch to blood combinations. When cotton was the secondary substrate, both touch-to-blood and blood-to-touch transfers were higher under friction contact. However, with plastic as the secondary substrate, the opposite trend was noted for both touch-to-blood transfer scenarios (plastic and cotton primary substrates). It has been documented in several studies that friction contact can result in blood flaking and dislodgement [4,9], especially from smooth surfaces. In touch-to-blood transfers on plastic, it is possible that while friction initially increased DNA transfer to the blood-covered substrate, this transferred DNA was subsequently transferred back to the original substrate (or lost), along with the top layer of blood flakes created by the frictional contact. In blood-to-touch transfers, we observed different patterns depending on the substrate materials involved. For plastic-to-plastic transfers, the same trend of lower transfer under friction was noted, likely due to blood flaking from both substrates. This flaking probably equalized the DNA amounts between the two plastic substrates. In contrast, for cotton-to-plastic transfers, friction increased the transfer of blood, although not to a statistically significant degree. This was comparable to unidirectional transfer of touch and blood where friction contact significantly increased transfer rates [4,5].

Independent of contact type, substrate characteristics play a significant role in transfer events. Highset transfer rates, for bi-directional

Table 4
Mean % Transfer of DNA (SD) between primary and secondary substrate combinations under passive and friction contact for bi-directional transfer of touch DNA to blood covered (BD-TB) and touch covered (BD-TT) substrates and unidirectional touch transfer (UD); * identifies significant bi-directional and unidirectional substrate combinations (K-W; $p < 0.05$).

	Secondary Substrate											
	Plastic						Cotton					
	Passive			Friction			Passive			Friction		
Primary Substrate	BD-TB	BD – TT	UD	BD – TB	BD – TT	UD	BD – TB	BD – TT	UD	BD – TB	BD – TT	UD
Plastic	10.6 (21.1)	4.42 (7.24)	2.7 (6.6)	0*	11.32 (20.84)	29.34 (30.7) *	3.66 (7.3) *	0.27 (0.75) *	18.46 (19.9) *	23.41 (7.3)	40.78 (38.15)	14 (18.59)
Cotton	10.2 (20.4)	0.20 (0.41)	0.28 (0.5)	0*	0.65 (1.57) *	7.9(3.9) *	0	0.56 (0.82)	2.07 (2.32)	12.82 (15.9)	24.70 (19.92)	32.55 (2.07)

Table 5
Mean % Transfer of DNA (SD) between primary and secondary substrate combinations under passive and friction contact for bi-directional transfer of blood to touch covered (BD-BT) substrate and unidirectional blood transfer (UD); * identifies significant bi-directional and unidirectional substrate combinations (K-W; $p < 0.05$).

	Secondary Substrate							
	Plastic				Cotton			
	Passive		Friction		Passive		Friction	
Primary Substrate	BD-BT	UD	BD – BT	UD	BD – BT	UD	BD – BT	UD
Plastic	0.02 (0.02)	1 (0.03)	0 (0)*	44.5 (0.1)*	0 (0)	0 (0)	0.02 (0.02)*	16.1 (0.1)*
Cotton	0.07 (0.15)	0 (0)	0.35 (0.67)	0.05 (0.001)	0 (0)	0 (0)	0.83 (0.77)	0 (0)

touch deposits, were from plastic as primary substrate to cotton secondary substrate. While for bi-directional blood deposits, the highest transfer was from cotton to cotton. In unidirectional transfer of touch and blood DNA, fresh touch samples, that transferred immediately upon deposit, and dried blood samples resulted in greatest transfer when both substrates were cotton, while dried touch deposits followed the bi-directional transfer pattern with highest transfer recorded for plastic to cotton substrate combination. Notably, cotton secondary substrate seems to result in increased transfer for both types of transfer. In general, the non-porous and non-absorbent nature of plastic allows cells to remain on the surface of the substrate, readily available for transfer. Cotton, in contrast, is a porous and absorbent material with a complex fibrous structure [7,22–24]. The rough fibres can effectively interact with a plastic substrate in two ways. First, they can dislodge loose DNA or cells present on the plastic. Second, once dislodged, these biological materials can become entangled within cotton's fibre matrix. This dual action of cotton – dislodging and trapping – enhances both the adhesion of DNA to the cotton and the transfer from primary substrates. Further, substrate chemical composition and interactions with biological materials can also play a role. It is likely that different substrates, with different porosity or substrate make up (fabric weave and weft and fibre composition) would absorb and interact with biological material differently and result in different transfer rates to those reported here [7,23,24].

In bi-directional transfer, touch DNA samples displayed significantly higher transfer rates than dried blood. Goray et al [4] also noted that once biological fluids (blood and saliva) dry they transfer less than touch cells that have been allowed to dry for 24hrs. When comparing the transfer of touch biological material to a substrate covered in touch or blood deposit, differences were noted. Under friction contact, greater transfer was noted to touch deposit secondary substrate, sometimes significantly so. As noted, blood is prone to flaking and may have dislodged newly transferred touch DNA. With passive contact, the opposite trend was observed. With plastic substrates, dried secondary substrate blood deposits may have altered the surface topography allowing for more opportunities for cells to become trapped and adhere, while avoiding loss from friction flaking [25]. Additionally, blood contains various proteins and molecules that may promote touch cell adhesion and migration [26]. This trend was less obvious with cotton, as during drying process, blood absorbs into the substrate reducing amount available on the surface for cell-to-cell interactions. The interactions between biological materials on both substrates during a transfer event may explain the differences in transfer between unidirectional and bi-directional transfer. As the adherence dynamics of EDTA treated blood, as used in this study and many other studies, may be different from non-treated blood directly from a body [27], as per casework situations, future transfer studies involving blood should aim to use non treated blood where possible.

It should be noted that while this study aimed to faithfully replicate the early unidirectional experiments including substrate and contact type and deposit amounts, the sample processing methods differed between the studies. In the Goray studies [4,5], older and less sensitive kits were used comparative to the present study. Intuitively, it may have been expected that higher transfer rates would be observed with touch deposits in the current study, owing to the superior sensitivity of the profiling kits used, yet this was not the case. However, the deposit area in this study was smaller (1 cm x 1 cm) to what was used in Goray et al (4.5 cm x 6.5 cm) [5] possibly offsetting the sensitivity differences.

This study demonstrates that the bi-directional transfer of biological material between substrates is influenced by the type of biological material present on the secondary substrate. While it is essential to consider the types of substrates involved, we must also examine how secondary biological materials can modify substrate characteristics and how different biological materials interact with one another. In a unidirectional transfer scenario, touch DNA adheres to a substrate based on the porosity, roughness, and chemical properties, such as wettability and hydrophobicity [7], and is different from how other biological materials such as blood, semen and saliva adhere [7,24]. However, the presence of biological material already adhered to the secondary substrate may alter these physiochemical interactions. This study has examined several variables that may be relevant during a transfer event. However, our understanding of these factors would benefit from repeating the experiments with a larger number of replicates, as indicated by the high standard deviation of results. Additionally, future studies should also consider testing a wider range of variables such as different substrates, biological materials and contact types. Future research should focus on understanding the interaction dynamics between two biological materials and how this interaction may affect bi-directional transfer processes.

5. Conclusions

There has been no published research with the intent of investigating the bi-directional transfer of DNA and directly comparing it to unidirectional DNA transfer results, under semi-controlled conditions. When comparing unidirectional data generated in Goray et al [4,5] to the bi-directional data generated through this project, it was identified how the same sets of variables impact both types of directional transfer. Results of this study support previous research that show transfer of DNA, in most circumstances, is best facilitated from a non-porous primary substrate to a porous secondary substrate [4,5,28]. Moreover, friction contact significantly increases transfer rates regardless of substrate type and biological material [4,5,8]. The bi-directional transfer of biological material was shown to depend on the biological material covering secondary substrate. When comparing unidirectional and bi-directional transfer rates overall, there is no significant difference between the two transfer types. This research provides baseline information on the bi-directional transfer rates of touch and blood biological material under semi-controlled conditions, building on previous research of unidirectional transfer.

6. Ethics statement

All sample were collected with informed consent and human ethics approval. This project was approved by the [Intentionally Left Blank].

CRediT authorship contribution statement

Georga Sallows: Data curation, Investigation, Formal analysis. **Duncan Taylor:** Conceptualization, Resources, Supervision, Writing – review & editing, Methodology, Formal analysis. **Roland A.H. van Oorschot:** Conceptualization, Resources, Supervision, Writing – review & editing, Methodology. **Mariya Goray:** Conceptualization, Resources, Supervision, Writing – original draft, Methodology.

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Declaration of competing interest

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

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scijus.2025.101248.

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