forensic sciences

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Review

The Transfer, Prevalence, Persistence, and Recovery of DNA from Body Areas in Forensic Science: A Review

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Abstract: Forensic and medical examiners are often required to sample the body of a victim (either living or deceased), or a suspect of a criminal offence, for foreign DNA. This can provide useful information when the alleged activity involves the presence of various bodily fluids such as blood, semen, and/or saliva, as well as skin contact made between a perpetrator and a victim. Optimal recovery techniques for the collection of DNA evidence, following crime-relevant skin contact, can be dependent on the surface being sampled. Additional factors to consider include the body areas typically contacted during various activities and the likelihood of non-self-DNA being present in those areas prior to contacts of interest. Therefore, an understanding of DNA transfer, prevalence, persistence, and recovery on a body can aid in the interpretation of DNA results given activity-level questions and increase the value of the findings from this type of evidence. This review aims to summarise research on DNA-TPPR concerning various human body surfaces following different types of activities. This review examines the prevalence of background DNA on different skin surfaces, the reported DNA transfer associated with different forms of contact, and how different cofounding factors can affect the persistence of DNA.

Keywords: background DNA; transfer; prevalence; persistence; recovery; trace DNA; skin surfaces; body sampling

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1. Introduction

Trace DNA can originate from bodily fluids such as saliva, semen, blood, or epithelial cells that are present in small quantities [1–4]. Depending on the case scenario, this DNA can also be degraded or inhibited. However, advancements in forensic DNA analysis, including multiplexing, enhanced sensitivity of PCR amplification, and direct PCR, have greatly enhanced our ability to generate DNA profiles from such samples. Modern profiling techniques can produce full DNA profiles from trace samples containing as little as a single cell [2,3,5–9]. The investigation of trace DNA, its biological source, and how it transferred to a target surface bares great interest to the forensic community and has numerous implications within forensic casework [4,10,11]. This information can be used to evaluate activity-level propositions, which have become increasingly common over the past decade [4,12–14].

The increased sensitivity of DNA technologies raises important questions regarding how DNA came to be on a target surface, object, or individual (transfer) [10,11,15–19]; what

biological sources were present prior to a criminal action (prevalence) [17,20–22]; what factors could influence the persistence of that DNA between deposition and sampling (persistence) [15,23,24]; and what sampling techniques can optimise results and reduce the loss of DNA during recovery, packaging, and analysis (recovery) [10,20,25–35]. DNA-TPPR is not only relevant in relation to a wide range of items that may be involved in criminal offences, but also body surfaces where physical contact between a victim and offender may occur.

Depending on the type of offence, forensic investigators or medical examiners may be required to sample the skin of either a victim or a suspect in an area where contact has been alleged [36,37]. This could include penile, vaginal, anal, or oral swabs, fingernail scrapings, hair combings, and the swabbing of other skin surfaces, such as the breasts, neck, arms, etc. [36,38,39]. Research has demonstrated the ability to obtain DNA profiles from skin surfaces of a victim, following the transfer of DNA by a mock offender [36,40]. There are various factors that can influence the probability of an offender's DNA being detected following skin contact, including an individual's ability to deposit DNA from a hand or other area of skin (i.e., their shedder ability) [41–43]; how much DNA was present on a targeted skin surface prior to contact (background DNA) [10,11,17,44]; the proportion of self-DNA to non-self-DNA on both an offender and a victim (including the non-victim DNA that was already present on the skin of the victim, and the non-offender DNA present on the offender prior to contact) [11,16,17]; the factors that might impact the persistence of biological materials across different body sample types [10,11,45]; and the efficiencies of collection methodologies and DNA extraction methods [10,37,46,47]. Much of the existing research addressing the ability to detect and recover transferred DNA following a crime scenario involves objects made of wood, glass, plastic, etc. [16,30,48–50]. The applicability of this research to predicting the recovery of DNA, specifically the non-self-component, from human skin surfaces allegedly contacted during a crime, is limited.

This review aims to provide an overview of research focused on addressing questions related to the transfer, prevalence, persistence, and recovery of DNA from skin surfaces relevant to forensic casework. By assessing the results of both external and internal body samples, this review aims to provide an understanding of the following key areas:

1. The role of sampling various body areas: how sampling different areas of the human body, that may have been contacted during a crime, can aid in investigations of various casework scenarios;
2. An overview of current data on the prevalence of background DNA on different skin surfaces, and its potential impact on the detection of DNA transferred during physical contact;
3. An examination of factors that can influence the transfer and persistence of DNA (both touch samples and various biological fluids) to different human skin surfaces;
4. A summary of recovery methods used for the collection and processing of biological samples from a body for the purpose of DNA analysis;
5. A discussion of the limitations in the current research that address the needs of forensic caseworkers and legal deliberators, along with suggestions for future research directions.

The methods applied to extract DNA from collected biological material, including samples collected from the body, to generate and interpret DNA profiles are not within the scope of this review. The papers that have been included in this review address the current knowledge surrounding DNA-TPPR from body samples, highlighting both established knowledge and areas requiring further investigation. Figure 1 outlines the key references discussed in this review, indicating the specific body areas that are the focus of each of those papers. It should be noted that the present review is not a discussion on body samples obtained for the purpose of identification (such as those that would be obtained in

a disaster victim identification exercise), but research involving the collection of skin/body samples with the aim of assessing DNA-TPPR relevant to a criminal offence involving contact between a victim and an offender. Furthermore, this review focuses predominantly on research investigating DNA-TPPR on external skin areas commonly contacted during various offences. However, it will also address internal sampling of the human body relevant to forensic casework. The information will be presented based off of targeted body areas, progressing from the head to the toes. Figure 1 also provides a guide for readers, indicating which numbered section of this review details the data for the relevant body part. This review aims to contribute to a broader understanding of how body samples are collected and evaluated in forensic science and identify areas requiring further research to strengthen their evidentiary value and reduce the challenges faced by forensic agencies.

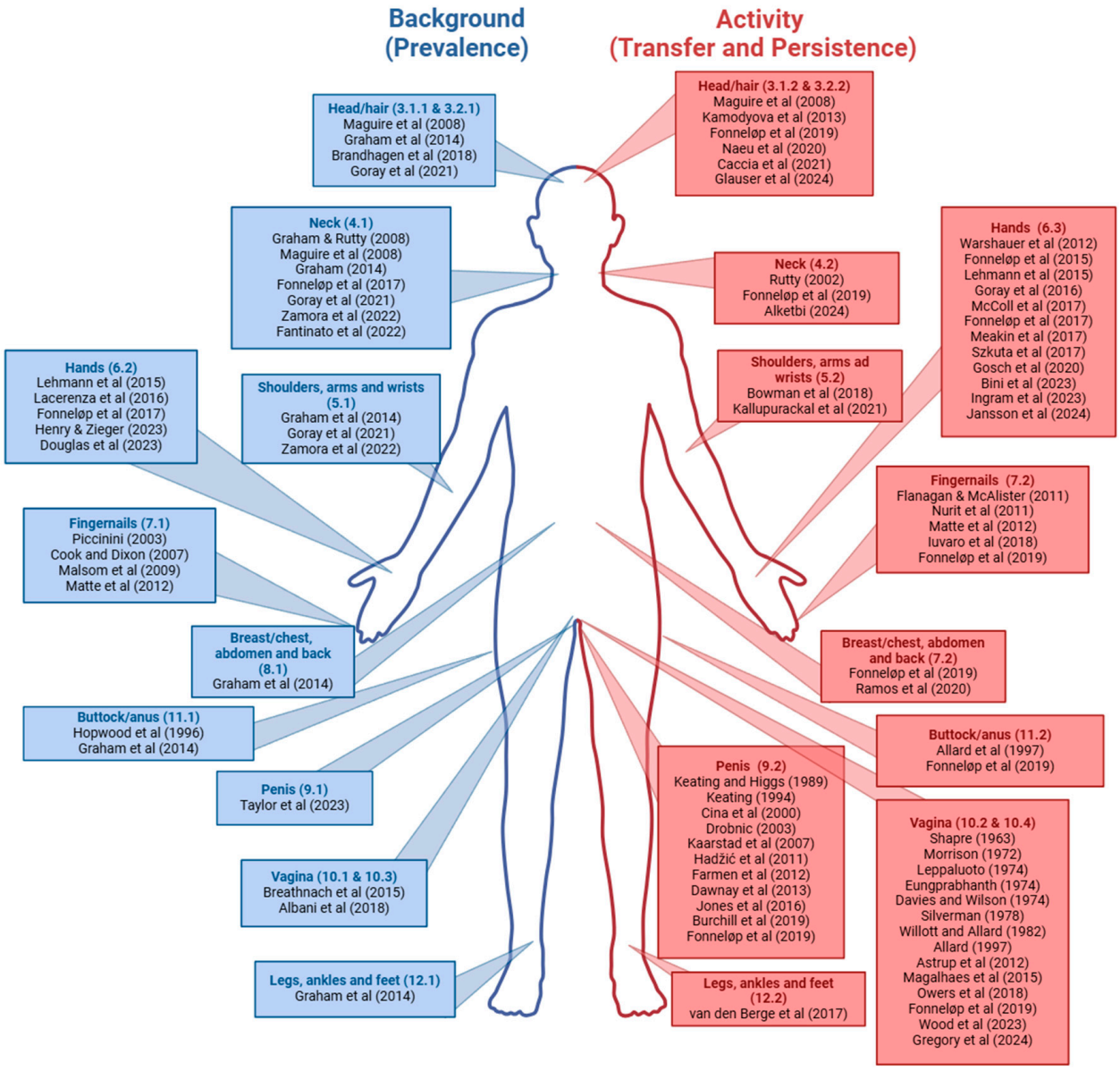

Figure 1. Summary of key references investigating the prevalence of the background, transfer, and persistence of DNA on different body parts and skin surfaces. References included are listed chronologically and are directly relevant to sampling of the specified body area. The relevant section number within the manuscript is indicated at the top of each box. However, additional relevant research is explored in each section [6,16–18,22,25,36,40–42,44,45,51–99]. Figure created in biorender.com.

2. DNA Recovery from Body Parts

2.1. Sampling Strategies

The strategy used to collect a trace sample at a crime scene or from an exhibit can determine the quality of a DNA profile obtained [37]. The same considerations must be made when determining the most appropriate sampling strategy for DNA recovery from skin surfaces of victims and/or possible offenders. The two primary collection methods for sampling skin surfaces are swabbing and tape lifting [11,100]. However, the appropriate method of collection is dependent on numerous factors, including the surface being sampled, the composition of the DNA (i.e., is it a biological fluid or touch sample), if the sample is wet or dry, and the body area targeted for sampling. Below, various DNA recovery strategies are summarised to provide an overview of commonly used methods and the relevant literature related to sampling in forensic casework, including techniques for obtaining samples from a human body. These techniques are also summarised in Table 1.

2.1.1. Swabbing

The double-swabbing technique evaluated by Pang et al. [101] is considered one of the most effective methods for the recovery of touch DNA samples from crime scenes and is widely utilised in numerous studies and forensic casework [25,101–105]. Kallupurackal et al. [25] compared various collection methods for recovering touch DNA deposited following skin-to skin contact whereby a male "offender" gripped the forearms of a female "victim" in a mock assault scenario. This study explored the use of Cap-Shure sterile cotton swabs (Puritan®) for single-swabbing methods using five different movements (wet tip with both rolling and rubbing movement replicating what is typically used in casework, wet tip with rolling movement, dry tip with rolling movement, wet tip with rubbing movement, and dry tip with rubbing movement); wet and dry double-swabbing techniques with Cap-Shure cotton swabs (Puritan®) and the use of nylon FLOQ swabs (Copan) for wet and dry swabbing with rolling and rubbing movements; and the tape-lifting method using SceneSafe FAST™ minitape (SceneSafe) [25]. The double-swabbing method recovered approximately 13.7% more offender DNA than other methods, suggesting double-swabbing techniques to be the most effective, regardless of the differences in the swab type used [25]. This sampling method has been successfully applied to a range of different body areas, due to its efficacy of targeting suspected trace evidence and its non-destructive nature, making it suitable for sampling of the human body.

Table 1. Summary of DNA recovery methods used in forensic science for collecting biological samples from a human body.

Technique	Body Part Commonly Sampled	Description of Sampling Technique	Relevant Papers Mentioned in Review
Swabbing	External skin surfaces (i.e., skin of the face, neck, arms, legs, external surface of the penis, external area of the vagina, etc.) Internal body areas (i.e., oral cavity, vaginal cavity, penis, and anus)	Double-swabbing technique is commonly used for external samples, whereby a wet swab is applied to the sampling area, followed by a dry swab. Alternatively, a single wet swab has been shown to yield similar results. Internal samples are also typically obtained with a single swab. Swabs are generally made out of cotton or viscose.	[25,86,101–103]

Table 1. *Cont.*

Technique	Body Part Commonly Sampled	Description of Sampling Technique	Relevant Papers Mentioned in Review
Tape lifting	External skin surfaces (i.e., skin of the arms, neck, chest, legs, etc.)	Used by applying the adhesive side of tape to the target surface (including skin) to collect traces of deposited DNA. This process has been carried out on the skin of live and deceased victims.	[25,26,46,106–109]
Hair/pubic combings	Head and pubic hair long enough to be combed	Involves using a fine comb to brush through hair and collect biological traces onto a piece of paper or collection device placed underneath the sampled surface.	[110–116]
Fingernail scrapings/ clippings	Underneath the fingernails	Achieved by either clipping of the fingernails or using a swab or scraping device to remove any trace evidence from underneath the fingernails.	[59,75,117–121]
Mouth rinses	Oral cavity	An individual rinses a solution (generally a saline solution) in their mouth, which is then deposited into a collection device.	[105,122–127]
Imprints	Teeth, skin (e.g., ear, penis)	A mould is used to recover an impression (typically dental), or a glass plate or smooth surface can be used to recover an impression upon contact.	[83,128–130]

2.1.2. Tape Lifting

Swabbing, as a method of DNA recovery, is primarily used to sample skin surfaces that may contain trace samples of DNA [26,66,72,131]. However, other collection methods, such as tapelifts, may be more suitable depending on the targeted biological source and the collection of hairs or fibres. Kenna et al. [26] investigated the persistence and recovery of 50 µL of saliva deposited on skin and found that the use of minitape produced better results in terms of DNA quantity recovered compared to swabs (15.77 ng and 12.71 ng recovered from tape lifting and swabbing, respectively) Furthermore, Hymus et al. [107] compared six different tapes, including Scotch® 183 Wall Safe Tape (3 M), Scotch® 502 Sticky Tape, Scotch® 600 Transparent Tape (3 M), Scotch® Super-Hold Tape (3 M), Lovell Surgical Solutions Clear Tape (Lovell Surgical Supplies International Pty Ltd., Bayswater, VIC, Australia), and Sellotape Strong Sticky Tape (Sello) (Henkel, Cheshire, UK) [107]. This study involved a participant brushing their body down with a lint brush after showering, and then submitting the brush to be sampled via differing tapelifts [107]. Across the tapelifts assessed, it was established that strong adhesion tape (Scotch® Super-Hold Tape) will not necessarily optimise the recovery of DNA, as a less-adhesive tape (Scotch® 183 Wall Safe Tape) recovered DNA as effectively [107], and that tape-lifting methods can be useful for the recovery of biological materials from a range of surfaces, including porous surfaces (denim and chiffon) and skin surfaces [107]. Moreover, additional studies have yielded comparable results [28,106–109].

Verdon et al. [29] investigated the use of Scotch® Magic™ tape and Scenesafe FAST™ minitapes for recovering touch DNA deposits from four cotton flannelette fabrics. While not focussing on body sampling, this paper is an example of a broad range of existing research which highlights the importance of using an appropriate sampling method to optimise DNA yield from the substrate of interest. The Scenesafe FAST™ minitapes were found to recover a higher quantity of DNA and increased number of alleles; however, the amount of DNA recovered by both tapes was dependent on the number of applications to the fabric being sampled [29]. In general, an increase in DNA recovery was noted, up to 32 applications of

the tape, at which time a progressive decrease in recovery was noted up to the maximum of 64 contacts tested. Zorbo and Jeunaiux [46] demonstrated the usefulness of using 1:1 tapelift methods in murder cases, whereby tape is used to cover all of the accessible skin surfaces of the victim to recover trace evidence. This technique is often used to obtain traces of hairs and fibres on victims' bodies in Belgium [46]. The authors also recommended collecting additional samples from areas of interest using techniques such as swabbing, following the initial tape lifting, to optimise recovery [46]. However, more research should be conducted to establish which recovery method is most beneficial across diverse anatomical regions, to optimise evidence recovery protocols dependent on the area of the body targeted as well as the type, amount, and condition of the biological source targeted in forensic casework. Differences in optimal recovery methods may exist for various body areas, influenced by factors such as the presence of mucus, oils, hair, and calluses; roughness; the rate of cell renewal and sloughing; the relative quantities of self and non-self-DNA due to the aforementioned reasons and/or clothing worn, environmental exposures, personal habits, and/or activities conducted; and anatomical accessibilities. Identifying the optimal recovery method for each area and circumstance would help forensic practitioners improve the likelihood that collected samples will assist in investigations.

2.1.3. Hair/Pubic Combings

Hair can be sampled following an offence in an attempt to recover various chemical and biological traces, typically pubic hair transferred from a perpetrator to a victim [39,115,132]. This form of evidence is usually recovered by combing through the head or pubic hair of a victim or alleged offender, making it a recommended recovery method specific to body hair [110]. The ability to recover traces of materials like pollen [111] and gunshot residues from hair [112,113,116] via combings has been reported previously; however, there is a lack of literature on the ability and best methods of the recovery of human biological material from hair. Naue et al. [114] tested four different methods for their ability to clean biological materials/stains from hair shafts. The following methods were explored: (1) 500 µL of DNA-free water; (2) 500 µL of a lysis buffer ("Buffer AL", Qiagen, Hilden, Germany); (3) a DNA-free swab moistened with 0.5% SDS; and (4) 3% NaClO (Roth, Karlsruhe, Germany) [114]. The swabbing method was found to be most effective at removing biological stains from the hair shaft without compromising the DNA for analysis, producing a full profile in approximately 66% of samples [114]. While this paper indicates the ability to recover biological materials from hair samples, it does not discuss the use of combing or other recovery methods and their potential use in forensic casework, highlighting the need for further research assessing the efficacy of head and pubic hair combings for recovering biological traces containing DNA. It should be noted that there is further literature that explores the ability to process hair samples for DNA analysis; however, this review only explores this briefly below (see Section 3).

2.1.4. Fingernail Scrapings/Clippings

Fingernail samples are typically collected by swabbing the underside of the fingernail or by using a wooden or plastic applicator to remove any trace materials (scraping) [117]. Alternatively, fingernail clippings can also be collected [39,117,132]. These samples are then processed in an attempt to detect any foreign DNA that might have transferred to underneath the fingernails [117]. Fingernail scrapings have been shown to yield sufficient DNA for genetic profiling in the forensic investigations of assault offences [59,75,119–121]. A retrospective study of 137 homicide cases presented by Nurit et al. [120] found 30% fingernail scrapings obtained from victims to reveal mixed profiles. Song et al. [118] discusses the analysis of fingernail clippings recovered from a suspect alleged to have strangled

a women. The fingernail clippings revealed the victim's DNA to be present, despite being recovered two days following the offence [118]. A comparative study conducted by Hebda et al. [117] compared fingernail soaking, scrapings, and swabbing (wet and dry swabbing using compressed CleanFoam® swabs) as methods of DNA recovery following assault scenarios. This research utilised Y-STR and autosomal DNA analysis to compare the efficacy of exogenous DNA recovery via each collection method [117]. Following STR analysis, the nail clippings were found to recover 98% of exogenous DNA compared to 61% from swabbing and 33% from scraping, making nail clippings the recommended method of recovery when targeting DNA underneath the fingernails [117].

2.1.5. Mouth Rinses

A sampling strategy used for the collection of foreign DNA from an individual's oral cavity is mouth rinses or mouthwashes, with the primary aim of recovering DNA that has transferred to the oral cavity during an offence [125,133]. This type of DNA recovery method is a non-invasive approach of collecting DNA from the oral cavity of an individual, typically with a saline rinse [105,122,124–127]. Heath et al. [125] investigated the use of different mouthwashes for the purpose of collecting DNA, including Cepacol, Listerine, Listermint, Scope, and saline mouth rinses. Each wash was ranked on their compatibility with DNA purification chemistry, DNA yield, the quality of the DNA obtained, the DNA's stability in the mouthwash, and its taste [125]. These criteria were used to rank the mouthwash type tested, leading to the conclusion that the Scope mouthwash was most suitable and hence the recommended mouthwash for this recovery method. The Scope mouthwash was then validated for its ability to recover DNA, yielding between 12 and 60 µg of DNA from 14 samples analysed [125].

Feigelson et al. [122] obtained Scope mouth rinses from twenty-four volunteers submitted on six different days, to assess the effect of rinse time, toothbrushing, and time between collection and extraction on DNA quality and yield. In the study, toothbrushing up to 1 h prior to sample collection reduced the amount of DNA recovered by up to 40% [122]. The DNA quality and yield remained optimal for up to 5 days between collection and processing, extracting an average of 32 ng of DNA. Garcia-Closas et al. [123] explored the use of buccal cytobrushes and alcohol-based mouthwashes for collecting DNA from the oral cavity, revealing better performance of mouthwashes (median DNA recovery of 27.5 µg/mouthwash) in comparison to cytobrushes (1 µg/two brushes) [123]. Roberts et al. [134] found that flossing could be used to retrieve sperm in oral sexual assault cases, and that after increased time since intercourse (>12 h) flossing exhibited a higher success rate than oral swabs. Much of the research related to the collection of DNA from mouth rinses has been conducted to improve methods of DNA collection for the purpose of haplotype determination [124], genetic analysis [105,127], and other clinical applications not specific to forensic science [122,123,125,133]. However, this technique can also be applicable to forensic casework.

The extent of DNA transfer, persistence, and recovery of saliva is dependent on a sample being wet or dry [49,135,136]. As Thornbury et al. [137] observed, saliva deposited while chewing gum did not dry as expected from normal saliva deposits. Such factors should be considered when saliva is the source of biological material of interest. While the impact of wet versus dry saliva samples on DNA profiling has not been thoroughly explored, existing research suggests that wet saliva samples, whether recovered from mouth rinses or chewing gum, can be effective for generating DNA profiles.

2.1.6. Imprints

Imprints or impressions can identify marks left on the skin by a known item [130]. Dental impressions can be compared to a bite mark left behind on skin or another object following an offence [128,130]. While dental impressions are not typically targeted for the purpose of DNA analysis, Anzai-Kanto et al. [138] demonstrated the ability to recover full DNA profiles from saliva deposited on skin. Other imprints investigated briefly in forensic research include ear [129] and penile imprints [83] collected by pressing the targeted area onto a glass slide [83,129]. The markings can be used for identification and/or for the collection of DNA for genetic profiling. Kaastard et al. [83] conveyed the usefulness of penile imprints, obtained during sexual assault offences, for the recovery of female DNA. In their study, 26% of all samples contained DNA from the female victim. No data are currently available addressing the process of recovering DNA from an ear print for the purpose of DNA profiling; however, the literature indicates the ability to use ear prints as another form of evidence [129].

2.1.7. Sampling of Areas Adjacent to Target Areas

As areas of skin targeted for sample collection will also likely contain background DNA, it is therefore pertinent to consider how the presence of background DNA might influence the generation of a DNA profile. This background DNA usually includes the DNA of the person being sampled but may also include non-self DNA of known and unknown sources. Awareness of the composition of background DNA detected adjacent to the target area has shown the potential to improve discrimination power within the targeted sample [139,140], and should be considered during external body sampling.

3. Head and Hair

3.1. External Areas

3.1.1. Head and Hair: Prevalence of Background DNA

Typically, DNA is obtained from the root of the hair, which contains nuclear DNA (nDNA). However, in the absence of a hair root (which is common in shed hairs), DNA, presumably from epithelial cells and oils from the scalp, can still be recovered from the hair shaft and used for profiling [52]. Alternatively, mitochondrial DNA (mtDNA) can be targeted from the hair shaft for profiling; however, its limited discriminatory power presents certain disadvantages [114,141]. Investigations into the recovery of non-donor DNA from human head hairs have not yet been explored. However, the ability to recover sufficient human DNA from the fur of pets has been recently demonstrated, suggesting the possibility of DNA recovery from human hair [142,143]. Hence, this knowledge would be valuable in forensic casework.

Several studies briefly explored the ability to detect background levels of DNA from the surface of a face [44,51]. Maguire et al. [51] first explored the prevalence of background DNA on the facial skin of children, among other skin surfaces. This study primarily focussed on the feasibility of swabbing a child's face to recover DNA without causing distress [51]. Thirty children were sampled from twelve different face regions and approximately 30% of samples exhibited non-donor DNA. However, only partial non-donor profiles were detected (1–2/10 alleles) in combination with the full profiles of the donors [51]. It is important to note that this study used an SGM Plus amplification DNA kit and newer, more sensitive DNA kits may result in enhanced detection and discrimination, and this should be researched in further studies.

A follow-up investigation by Graham et al. [44] generated further data on the levels of background DNA typically detectable on the faces and other body parts of children. A total of 944 face/neck and body samples from 47 volunteering children were collected for

analysis [44]. In general, more self and non-self alleles were detected from the face than the body. Across all face/neck samples obtained, on average 12.5 of the child's alleles (67% of full profiles), and 0.99 of non-child alleles were detected. The skin from the front of the hands and cheeks of children recovered the most foreign DNA [44]. No significant effects were noted for age, gender, development (pre- and walking), feeding (breastfeeding and other), the use of cream, washing, and being kissed on the recovery of foreign DNA. There is currently no existing research which addresses how different forms of contact (i.e., hugging, general face touching) and general contact between individuals can influence the prevalence of background DNA on the faces of children or adults, and how this can change following a criminal offence where forceful contact can be made. Little has been reported on the background levels of DNA on adults. Goray et al. [53] sampled the foreheads of four individuals via double swabbing to establish the relationship, if any, of samples from these surfaces with the shedder status of an individual. Two samples were obtained from each individual, whereby one participant revealed an average of 1.5 contributors, with donor DNA making up 95% of the profiles. The other three participant samples produced single-source profiles of the donor (DNA quantities ranging from 27.3 ng to 96.9 ng) [53].

Further research should be considered to establish background DNA levels, and origins, on the faces of adults and children, within different living, working, and social environments, with different habits, and after a wide range of common activities.

3.1.2. Head and Hair: DNA Transfer and Persistence After Activity

There has been minimal reporting on the transfer and persistence of foreign DNA to and on hair shafts or the surface of head hair. Naue et al. [114] investigated the recovery of donor DNA from hair shafts contaminated with different biological materials, including blood, saliva, semen, vaginal secretions, and skin cells, using different cleaning methods. The ability to effectively remove a biological stain from a contaminated hair shaft, preserving the integrity of both, allowed for both to be profiled while reducing the chance of mixtures [114]. Caccia et al. [115] investigated the detection and persistence of semen and blood on hair exposed to outdoor environments using microscopy and common chemical tests. Hair was donated by a female, and the samples were prepared by cutting approximately 15 cm sections of hair followed by sample deposition. Post-deposition, samples were exposed to a natural outdoor environment (woods), manmade outdoor environment (roof), and controlled environment (inside the box) for three months with regular test intervals. For semen detection, PSA analysis, macroscopic observations using a Crime-lite® lamp (Foster + Freeman, Worcestershire, United Kingdom), and Sperm-HY-LiterTM Express (Independent Forensics, Chicago, Illinois, United States) were used, while blood was tested with Combur3Test®E, Hexagon OBTI, and Luminol [115]. Three months post-deposition, blood was detected with all three chemical tests, while semen was observed for up to a month with Sperm-HY-Liter, and PSA testing was positive for up to 1 week in both environments. From a total of 30 samples analysed, the results revealed that 50% of profiles obtained from blood or semen stains on hair shafts collected from different environments after various time periods were complete STR profiles, with partial or negative profiles being less frequent (26% and 23%, respectively) [115].

The propensity for individuals to touch their faces (both consciously and subconsciously) is an aspect of human behaviour that can directly influence the transfer and persistence of biological material containing DNA [144–147]. Kwok et al. [145] conducted an observational study, recording the frequency of face-touching behaviours among medical students, finding that each individual touched their face an average of 23 times in one hour. This research was conducted to address the ability for pathogens to be transmitted to the face via the hands; however, it can be applied to the transfer of DNA to and from

facial surfaces in a forensic context [145]. Zacher et al. [147] addressed the ability for DNA to transfer from innocuous contacts between hands and faces of individuals during a one-hour-period that was video-recorded. The experimental design involved intruders entering participants' office spaces and performing different scripted and unscripted tasks without wearing gloves [147]. Five participants (two office owners and three intruders) provided face samples and the review of the video recordings showed that all participants touched their own faces during the experiment [147]. Foreign DNA was detected on two of the face samples. Apart from the donor, two other unknown contributors were detected. Of note, the three samples where only face donors were detected had much higher total amounts of DNA recovered (ranging from 14.8 to 110.5 ng of DNA) compared to samples where foreign DNA was detected (0.08 ng of total donor DNA detected in both samples; 0.09–0.1 ng of total foreign DNA) [147]. The authors indicate that the variations in shedder ability among participants may be partly responsible for their observations.

Fonneløp et al. [38] analysed 45 facial and 62 samples from the lips and around the mouth swabs collected from victims of sexual assault offences reported between 2013 and 2015; the POI's DNA detection was reported in 38% and 15% of the samples, respectively. While there is still limited forensic research specifically investigating the transfer and persistence of DNA on facial surfaces relevant to a crime, Jansson et al. [144] identified the influence that face-touching habits can have on the transfer of touch DNA. The touching of the face can provide additional avenues to introduce foreign DNA to the face and for DNA to be collected by an individual's hands for subsequent transfer to touched surfaces [144]. This may be dependent on the area of the face being contacted, e.g., areas where saliva may be more prevalent. Moreover, little is known about possible differences in the behaviour, including transfer and persistence, of different biological sources on facial surfaces. For example, the propensity of DNA from different sources to be transferred and persist on the lips, compared to the cheeks, chin, or forehead, of an individual involved in an alleged crime (i.e., a sexual assault where kissing might occur) may differ.

Other external surfaces of the face that may be of relevance during criminal investigation are the eyes and ears. While the eyes are not commonly sampled for DNA analysis, research has shown that tears can contain DNA, which can then be sampled and profiled [148,149]. Aparna et al. [149] collected tear-stained tissue and fabric substrates from twenty-five donors. Tears were induced with irritants such as menthol balms, exposing eyes to the air, and rapid eye movements, in an attempt to collect even volumes [149]. Additionally, ten donors wore non-prescription contact lenses, all of which were analysed. The results found that full profiles were obtained from all three substrates, with 100% allele recovery, conveying the success of gaining DNA from tears [149]. The transfer and persistence of DNA recoverable from other internal locations of the face has not been explored. However, the ability to transfer ear marks, and therefore DNA, has been investigated in several studies [150,151]. Meijerman et al. [151] reviewed different ear characteristics, as part of the FearID research project, aiming to identify a person's unique features. A variety of classification features from ear prints was collected, but the utility of these features is limited due to a lack of data, leading to false-positive identifications [151]. Graham et al. [150] explored the ability to transfer ear prints to a surface upon contact. This research involved the collection of ear prints from the right ears of 10 low, 10 intermediate, and 10 high shedders onto glass surfaces [150]. Donor DNA was detected in 75% of the samples (but only one full profile from a designated good shedder), while foreign DNA was detected in 33% of the samples and as a major contributor in 12% of those [150]. Collectively, these data suggest that face samples may provide relevant forensic information, but a better understanding of the issues related to DNA-TPPR is needed to place this evidence in proper context.

3.1.3. Internal Facial Cavities: Prevalence of Background DNA

The internal areas of the face include the oral cavity, nostrils, and ear canals. Of these body areas, the oral cavity is the most frequently sampled in forensic casework. This is because buccal cells are a rich source of DNA and provide a convenient and non-invasive source of self-DNA for use as reference profiles from known persons of interest [152,153]. This differs from oral samples, which are obtained through swabbing or mouth rinses following alleged oral assaults (e.g., oral intercourse, forced kissing), where foreign DNA transfers to the oral cavity, which will be discussed below. While there are no studies addressing the prevalence of background DNA from foreign sources in the oral cavity, common sense suggests the low persistence of non-self-DNA from the constant renewal of self-DNA, and the removal of both self and non-self-DNA, through the digestive tract.

Other internal areas of the face/head that contain biological materials for sampling include the inner ears and noses of individuals. These areas are not typically sampled during forensic casework, and there are no data available on the prevalence of background DNA among these body areas. However, research has shown the ability to generate genetic profiles via STR analysis from biological materials such as earwax [154] and nasal mucus [155]. Amer et al. [154] obtained sixty earwax swabs from male individuals that were stored for 1, 15, 30 and 60 days. Full STR profiles (15 loci,) were developed from the samples processed 1, 15, and 30 days following collection, with only 54.43% of the samples processed after 60 days producing an STR profile [154].

3.1.4. Internal Facial Cavities: DNA Transfer and Persistence After Activity

The direct as well as indirect transfer and persistence of saliva across a range of different surfaces has been reported in several studies [69,156,157]. However, the transfer of saliva or other biological materials to and from the oral cavity of an individual has not been investigated to the same extent. Saliva can transfer between the oral cavities of individuals through actions, such as kissing [158,159]. Kamodyova et al. [158] swabbed the mouths of women who partook in kissing with a male partner, collecting oral swabs immediately before kissing, then after 5, 10, 30 and 60 min time intervals [158]. Male DNA was identified through Y-STR profiling up to 60 min post-kissing, whereby the average number of Y-STR alleles detected from all participants decreased from 16 at 1 min post-kissing to 1.5 alleles detected after 60 min [158].

Through their research on the genetic profiling of earwax (as described above), Amer et al. [154] suggested the possible sampling of earphones and earplugs for earwax material that has been transferred during a criminal offence, and Seo et al. [160] recovered a full STR profile (using phenol–chloroform extraction) from a used earbud. To what extent self DNA is picked up by fingers while picking one's nose or ear and contributing to the quantity of DNA on their hand for further transfer, or the non-self DNA present on fingers being transferred to the nose or ear during such an action and persisting there, is unclear.

Glauser et al. [161] investigated the transfer of fibres to nasal cavities following acts of suffocation or similar actions. Due to fibres often being a source of DNA [149,161], there is potential for DNA to be transferred to such areas during a crime. It would be of interest to investigate if DNA can be recovered from the fibres transferred to the nasal cavity. This is further supported by recent studies showing that DNA can be aerosolised and present in the air for a period of time [162–164], and thus conceivably inhaled and trapped with other particles in the nasal cavity.

4. Neck

4.1. Neck: Prevalence of Background DNA

Non-fatal strangulation has been found to occur in approximately 58–67% [165] of domestic violence offences [40,165–167]. The "normal" levels of background DNA from non-self biological materials present on the necks of individuals, due to adventitious transfer throughout everyday activities, have been explored in the literature [40]. As a part of their shedder studies, Goray et al. [53] swabbed the back of four individual's necks, finding an average of 1.75 contributors detected. Graham and Rutty [40] sampled the necks of 24 adult individuals, finding non-donor DNA alleles in 23% of the samples, of which 5% had more than six non-donor alleles [40]. Expanding on this, Fantinato et al. [54] observed the persistence of non-self DNA on the necks of 20 adults over a 24 h period in relation to their daily activities, addressed through a questionnaire. Six samples were collected from each participant at different timepoints to assess DNA persistence [54]. Reference samples from partners and family members were obtained to establish the source of non-self DNA [54]. Samples from eleven participants (9%) detected unknown DNA in samples collected later in the day, following the use of public transport or attending a public setting [54]. These findings indicated an increase in the number of non-self alleles detected on necks over time or from exposure to the environment [54].

Zamora et al. [22] explored the prevalence of non-self-DNA on sebaceous skin surfaces of the upper arms (see Section 5.1.) and areas of the neck, specifically below the ear and on the sides as well as the nape of the neck. A total of 252 tapelift samples from 15 male and 13 female volunteers were collected in triplicate at least one week apart [22]. The time since last shower was recorded for each sample. The results showed that background DNA was detected in 3.6% and 9.6% of samples from below the ear and the neck, respectively. Furthermore, in 2.4% of these samples the donor was the minor contributor [22].

4.2. Necks: DNA Transfer and Persistence After Activity

It is important to understand the persistence of DNA on different skin surfaces following an assault scenario, to effectively evaluate the presence or absence of DNA following this type of contact. Alketbi [68] focuses on evaluating the persistence of touch DNA transferred during simulated strangulation, whereby the offender firmly gripped the neck of the victim for 2 min. An offender's DNA on the neck of a victim decreases over time, decreasing from 1:2 mixture ratios at 1 h to 1:13 after 48 h. No offender's DNA was detected 72 h after initial contact [68]. Similarly, Rutty et al. [67] investigated the recovery of an offender's DNA from the neck of a victim, detecting offender DNA in 37% of the samples that produced a positive amplification result with standard SGMplus protocols up to six hours post-contact. When a selection of these samples was subjected to LCN analysis, an offender's DNA was detected up to 10 days post-contact, mostly as partial profiles [67]. A more recent study by Fonneløp et al. [38] analysed 92 casework samples from the neck/throat of victims, detecting offender DNA in 33% of samples. It should be noted that both Alketbi [68] and Rutty et al. [67] identified challenges to detecting transferred DNA, as the persistence of DNA generally decreases over time due to a number of factors, such as subsequent contact with the target area, washing, and exposure to various environmental conditions [67,68].

5. Shoulders, Arms, and Wrists

5.1. Shoulders, Arms, and Wrists: Prevalence of Background DNA

Van Oorschot et al. [10] highlighted the importance of considering background DNA during the interpretation of DNA profiles from the collar or upper arms of a garment if forensically relevant contact is suspected during a criminal offence, such as being grabbed. This

is also applicable to suspected direct skin contacts made to these areas. Graham et al. [150] investigated non-self DNA present on the back of children's upper arms and the front of the upper right arm. On average, 0.43–0.9 alleles were found on all of the areas sampled, suggesting a low percentage of non-donor DNA detectable on the upper arms of children [150]. Goray et al. [53] collected samples from the upper and lower arms of four adult individuals to assess the amount of DNA detectable on these surfaces. In the study, 8 of the 16 samples produced two-person mixtures; however, donors were always detected as major contributors (>93%) [53]. Zamora et al. [22] also revealed higher instances of detecting mixed DNA profiles from the skin of the upper arm compared to the nape of the neck and below the ear. Their investigation into the prevalence of background DNA revealed that 58% of the samples from the arm were single-source profiles matching the donor, 14% of the samples were mixed profiles with the donor being the major contributor, and 11% of profiles were mixed profiles where the donor was the minor contributor and non-donor DNA the major [22]. While existing research on background levels of DNA on arms is limited, some inferences can be made by studying the levels of wearer and non-wearer DNA on areas such as the sleeves and other areas of clothing [56,168–170]. The general consensus from these studies indicates that a wearer's DNA is typically detected in high proportions (approximately 84% of the profile [170]) compared to non-wearer DNA, which can be detectable in minor quantities (typically less than 20% [168]); however, these studies are not discussed extensively in this review [56,168–170].

5.2. Shoulders, Arms, and Wrists: DNA Transfer and Persistence After Activity

The shoulders, arms, and wrists of a victim may be contacted by a perpetrator during a physical assault [25,36,171]. Bowman et al. [36] addressed this through simulated mock physical assault scenarios to assess the extent to which detectable levels of an offender's DNA were transferred to a victim and clothing. Participants, acting as offenders, grasped the wrists and upper arms of the participants posing as victims, first applying medium pressure and no friction, followed by heavy pressure and friction [36]. Samples from the victim's skin were collected using the double-swabbing technique immediately, 3 h, and 24 h post-contact by an offender [36]. The offender's DNA was detected in the majority of samples collected immediately after contact (66%), decreasing over time to 25% at 3 h and 12% at 6 h, and increased pressure as well as friction resulted in a higher transfer rate. Further, subsequent transfer to clothing resulted in detectable transfer in 19% of the samples. Fonneløp et al. [38] analysed 27 casework samples recovered from the arms of victims of sexual assault, detecting an offender's DNA in 11% of the samples.

Kallupurackal et al. [25] examined the efficiency of various recovery methods for collecting DNA transferred to the wrists and arms of individuals during directed contact. Touch DNA samples were obtained from the forearms of female "victims" following a mock assault through gripping/grasping of the arms by an "offender". Overall, 4–64% of the offender's DNA profile was obtained corresponding to mean amounts of offender DNA, 0.43 ng–2.7 ng, dependant on the swabbing technique used (also see Section 2.1.1 for more information on the recovery methods/swabbing techniques tested in this study). This highlights the importance of selecting an optimal recovery technique based on the sampling areas under investigation.

6. Hands

6.1. Shedder Status and Touch DNA

It is known that people can deposit DNA upon hand contact with a target surface, object, or individual [1,41,43,172–174]. Touch DNA is a term commonly used across forensic research and casework to describe the DNA deposited by touch [1,42,43,175,176]. This

concept was initially pioneered by van Oorschot and Jones [176], who established the ability to obtain full DNA profiles from touch DNA deposited on an item following hand contact. While touch DNA is primarily thought to consist of epithelial cells shed from the hands, contributions of cell-free DNA, sweat and oil secretions, and cells collected from contact with various body parts and personal items are also believed to be important [1,16,42,49]. While generally recovered in trace amounts, it can also be found in high quantities if transferred by someone who has a high ability to shed DNA [1,18]. How much touch DNA an individual tends to deposit upon hand contact is termed their shedder ability (or shedder status), a phenomenon that has been broadly explored in forensic research [1,41,43,172,173]. Shedder status is important when evaluating DNA findings at the activity level [53,173].

Bini et al. [71] investigated the effects of alcohol-based hand sanitizers on the transfer of touch DNA. Their experimental design involved twenty participants depositing fingerprints onto glass surfaces before and after using hand sanitizer. A subset of these samples involved saliva deposits onto the hand before sanitizer use [71]. With saliva samples, the use of sanitiser resulted in deposit decreases from 77.5 pg/µL to 16.5 pg/µL of DNA, 1 h after sanitizer use. Similarly, the touch deposits decreased from 19.8 pg/µL to 12.7 pg/µL of DNA [71]. While these decreases were not statistically significant, the authors concluded that alcohol-based hand sanitizers can reduce the DNA amounts transferred from the hand [71].

Goray and van Oorschot [42] tested participants' shedder ability (both self and non-self DNA) by placing their hand prints onto DNA-free glass plates across multiple days and timepoints. This research aimed at investigating the relationship between an individual's ability to shed DNA (both self and non-self) and the implications this can have on the detection of touch DNA at a crime scene [42]. These studies confirmed that people can be classified as poor/low, moderate/intermediate, or good/high shedders of DNA, and explored different methods of classification. Their research found (in one of the many metrics tested) that low shedders deposited low amounts, below 0.5 ng of DNA, while high shedders routinely deposited an excess of 4.5 ng of DNA [53]. Furthermore, their research also revealed non-donor DNA to be detected across many samples, with a higher proportion of non-donor DNA detected in samples deposited by low shedders. Their research also explored the possibility of direct body samples, including faces, arms, and hands, to be used in shedder testing [53]. However, the authors did not find a relationship between the amounts of DNA present on these body parts and a participant's ability to shed DNA upon contact [53].

There have been numerous other studies that explore shedder status and its implication for forensic investigations [41–43,53,71,73,144,172,173,175,177]. Furthermore, recent developments in shedder testing shifted the categorisation dialogue from using discrete categories to shedder distributions that are now believed to be more representative of shedder types in the population [178]. These papers will not be discussed at length in this review; however, when considering hand sampling and the assessment of touch DNA-TPPR from the hands, it is important to acknowledge shedder status and the previous research that has been conducted in this area.

6.2. Hand Samples: Prevalence of Background DNA

Several studies have examined the levels of self and non-self DNA present on an individual's hands [11,17,41,42,56]. Taylor et al. [179] demonstrated the importance that considering background DNA on hands can have to an evaluation of the findings in a DNA case. Fonneløp et al. [41] highlighted the implications that background DNA on the hands can have when evaluating DNA evidence. In that study, "attackers" made hand contact with the clothing of the "victims" and the proportions of self and non-self DNA

were assessed in light of their shedder status [41]. Their research found that levels of DNA transferred from an attacker increased from 58% to 95% between low and high shedders. However, the ability to detect an offender's DNA from a "high shedding" victim typically decreased due to the victim's higher propensity for pre-existing background DNA [41].

Lehmann et al. [17] highlighted the influence that background DNA can have in reducing the relative quantity of transferred DNA detected following touch contact. In their experimental setup (Experiment 1), a single source of background DNA (wet blood, dried blood, or touch DNA) was deposited onto six glass (hard non-porous) substrates. An additional sample of target DNA (wet blood, dried blood or touch DNA) was deposited onto the first substrate. This resulted in the surface containing two DNA sources, the target and the background. The first substrate was then placed on the second substrate (15 s with pressure), after which the second substrate was placed on the third substrate in the same manner, and so on until the sixth substrate. This same set of tests was repeated using cotton fabric (soft porous) as the substrate to investigate how the surface can affect the transfer of DNA when background DNA is also present [17]. While the majority of combinations of target DNA and background DNA did not significantly alter transfer rates, when compared to situations where no background DNA was present [180] there were some exceptions. For example, the greatest differences were observed when background DNA was either wet or dried blood, opposed to touch deposits. Across glass substrates, less wet blood transferred when background DNA was wet blood compared to no background; however, whether the transfer increased or decreased in the presence of background DNA was dependant on substrate and biological material combinations [17].

Lacerenza et al. [55] investigated the types and levels of biological materials typically present on an individual's hands through DNA and mRNA profiling. This research established the prevalence of saliva, blood, semen, mucosa, and vaginal mucosa on an individual's hands through mRNA-specific markers. One or more of these markers were present in 15% of the samples, and resulted in an increased median amount (5.1 ng) of DNA compared to samples where these markers were not detected (1.6 ng) [55]. A negative correlation was also noted between the percentage of foreign DNA detected and the overall integrity of the DNA profile [55]. The researchers proposed that the higher prevalence of foreign DNA on the hands of female participants resulted from the lower quantity and integrity of self DNA and the consequent preferential amplification of the non-self DNA picked up from the environment. Male participants, authors suggest, may have laxer hygiene habits and thus enhanced accumulation and detection of self DNA components [55]. Therefore, biological sex was also identified as a significant factor influencing the prevalence of self and non-self DNA on hands, as samples from the males yielded higher amounts of DNA (median of 3.6 ng) compared to females (median of 1.0 ng) [55]. The prevalence of background DNA from different body fluids detected on hands has also been explored by Douglas et al. [57]. A participant's hands were tested for the presence of saliva, blood, semen, hairs, fibres, and debris collected from the general population, following normal everyday activities [57]. The hands of 66 male and female participants were sampled using the double-swabbing technique, and a questionnaire was completed to establish their recent activities [57]. Following presumptive tests for body fluids and microscopy analysis, hairs and fibres were collected from approximately one-third of the samples, while 2% of the samples detected spermatozoa. Further, the majority (72%) of samples were found to contain amylase, suggesting the presence of saliva [57]. In contrast, blood and semen were not detected. These findings suggest the likelihood of detecting different biological materials, except for saliva, on the hands of individuals following everyday contact is low [57].

Henry and Zieger [56] also investigated the ability to detect self and non-self DNA from the hands and sleeve cuffs of individuals following everyday normal activities and during a criminal offence scenario [56]. Left and right hand samples from four individuals were collected at different timepoints throughout the day across 25 days, providing a total of 200 samples [56]. Generally, the quantity of self DNA detected on the hands of participants was higher compared to non-self DNA. However, 15.8% of the samples recovered higher amounts of non-self DNA than self DNA, which researchers explained by a high number of contributors being detected across a lower amount of recovered DNA. The researchers linked this to the shedder status of each participant, explaining that larger proportions of non-self DNA were deposited by poor shedders. These findings highlight the possibility for the transfer of non-self DNA (possibly in higher proportions to that of self DNA) present on an individual's hands during contact [56].

6.3. Hand Samples: DNA Transfer and Persistence After Activity

Investigations into the propensity to transfer DNA from hand contact during a criminal offence scenario have been broadly explored [6,11,41,67,70], particularly with the increased knowledge surrounding shedder ability and touch DNA [73]. Hands are likely to make numerous contacts over a short duration of time, impacting the relative quantities of various contributing sources acquired by the hands [181,182]. Research has been conducted to establish the frequency of hand contacts made in both a criminal and non-criminal context to inform how this can influence DNA transfer from the hands. Van Oorschot et al. [181] conducted an analysis of 160 videos, of approximately 15–40 s in length, of individuals performing common everyday activities (including eating meals, sitting at cafés, taking public transport, office multitasking, being at home, going to the park, and cooking) and identified that, on average, individuals made approximately 15 touches of both personal (including themselves) and non-personal items (including other individuals) every minute with their dominant hand, which varied depending on the type of activity. Stella et al. [182] conducted a similar study investigating the number of hand touches made during robberies and attempted robberies, analysing 47 videos approximately 40–100 s in length. This analysis revealed that the dominant hand of a perpetrator made contact approximately 5 times a minutes, and the non-dominant hand made contact approximately 12 times a minutes [182].

The amount of DNA deposited by a hand or conversely deposited onto a hand following contact varies depending on the parts of the hand making contact. McColl et al. [171] investigated the ability to transfer DNA to a hand, with the subsequent transfer of this DNA to another surface. This was achieved by participants placing their hand onto a glass plate covered with dried saliva, followed by contact with a DNA-free glass plate, to investigate secondary transfer from the hand [171]. Fourteen different areas of the hand were evaluated to identify variations in contact from different areas and their potential impact on DNA transfer [171]. Fingertips demonstrated the highest propensity to pick up (average of 21.87 ng) and transfer (0.36 ng) DNA to the glass plates compared to the remainder of the fingers and areas of the palm that picked up an average of 4 ng and 1.6 ng and transferred, on average, 0.06 ng and 0.25 ng of DNA, respectively [171].

Szkuta et al. [45] investigated the propensity for hands to directly transfer self DNA and indirectly transfer non-self DNA by having participants shake hands with their dominant hand under controlled conditions, followed by contact with glass slides both immediately and 15 min after a hand shake [45]. Mixed profiles were recovered in 89% of samples [45]. It was also found that the depositor was the major contributor in most instances, expect for 16% of samples collected 15 min following the directed hand shaking, whereby multiple other contacts were made between the handshake and deposition.

This aligns with the findings of Henry and Zieger [56], who reported non-donor DNA as the major component in only 15.8% of hand swabs. Further research conducted by Cale et al. [183] investigated the ability to have DNA secondarily transferred to the handle of a knife following a period of long hand holding between two individuals, with secondary transfer found to occur in 85% of instances. While this research contributes to the understanding of secondary transfer via hand contact, other researchers present commentaries on these findings, suggesting that this research utilised optimal experimental conditions conducive to increased transfer that were not reflective of real-life situations [183–185].

The transfer of DNA from other biological sources, such as body fluids, via the hand as a vector bares interest in some circumstances. Warshauer et al. [69] investigated various transfer events (primary, secondary, and tertiary) of saliva through hand contact with a pen and conical tube [69]. The researchers explored several transfer events, such as making contact between a thumb bearing saliva and a pen, which is then passed onto another individual who then contacts the conical tube. Comparisons of DNA amounts between direct deposits and secondary as well as tertiary transfer events found an 81% percent loss between primary and secondary deposition, suggesting lower possible transfer rates. It was also noted that, due to the lack of quantifiable results, tertiary transfer loss could not be estimated but is consistent with the finding of significant losses during the initial indirect transfer step. The overall findings indicate a reduction in DNA as the number of transfer events increases, aligning with the results reported by Lehmann et al. [17]. Collectively, these studies indicate that hands act as a vector for DNA transfer, influenced by various confounding factors [6,14,15,111,140,162].

Ingram et al. [72] explored the application of flow cytometry in processing samples collected from sexual assault offences where digital penetration occurred. Hand swabs from participants who engaged in digital penetration were collected at different timepoints. Some of the hand samples obtained were from participants who had washed their hands after digital penetration and prior to sampling, reducing the number of vaginal cells detectable. However, these samples were effective in establishing a sensitivity limit and posterior probability of detecting a cell type across samples where only trace amounts of biological materials might be present [72]. Furthermore, to investigate the identification of cells from samples containing a mixture of saliva and epithelial cells, participants were asked to insert their own fingers into the oral cavity [72]. Data on multiple cellular morphological features of epithelial, vaginal, or saliva/buccal cells were analysed [72]. In the study, samples collected after digital penetration resulted in a high posterior probability for vaginal cells (>0.90) compared to hand swabs collected without digital penetration, and the authors suggested reasonable differentiation between vaginal and saliva cells [72]. The use of flow cytometry provides a novel and promising way of addressing the question about the source of biological materials on the body, assisting in activity-level evaluations [186].

7. Fingernails

7.1. Fingernails: Prevalence of Background DNA

Another common form of biological samples collected in forensic casework is fingernail scrapings from a victim (live or deceased) or a potential suspect [18,39,187]. Various studies have been conducted to establish the general prevalence of background DNA underneath the fingernails of people in the general population [18,59–61,119,121]. Cook and Dixon [59] collected fingernail samples from both hands of 100 participants. In the study, 69.5% of the profiles obtained full or partial single-source DNA profiles, compared to 6% of mixtures. While not assessed in this study, the researchers suggested that several factors, such as age, sex, nail biting habits, cohabitation, etc., warrant further exploration

to understand their impact on the recovery of single-source or mixed DNA profiles from fingernail samples [59].

Malsom et al. [60] investigated the prevalence of background DNA (self and non-self) recovered from the fingernails of 12 individuals who cohabit with partners. Of the samples obtained in this study, 17% revealed a mixed profile, with the DNA of a cohabitant recoverable from under the fingernails. This contrasts with the findings of Cook and Dixon [59], where only 6% of samples produced mixed profiles. Thus, it is suggested that cohabitation can influence the prevalence of non-self DNA under fingernails [18,119].

7.2. Fingernail Samples: DNA Transfer and Persistence After Activity

It is suggested that different forms of contact can lead to varying amounts of DNA transferring to beneath the fingernails [75]. Iuvaro et al. [75] conducted a study involving deliberate scratching between 30 paired male and female individuals, whereby the females scratched the males in a mock assault scenario [75]. Foreign DNA from the male participant was detected in 95% of fingernail samples recovered from the female participants, decreasing to 60% approximately 6 h after the initial transfer [75]. Another study by Matte et al. [61] investigated the transfer of male DNA to underneath the fingernails of women in a controlled scratching experiment, to simulate the type of self-defence often observed during assault, and compared these findings to casework samples obtained from assault offences [61]. In the study, participants in one group lightly scratched the forearms of their partnered participant, while another group was instructed to vigorously scratch the forearms of the participants. Fingernail swabs were then collected immediately after scratching and at the end of the day [61]. Approximately 30% of the samples obtained immediately after light scratching revealed the male participant's DNA, compared to 40% of samples from those who were instructed to scratch vigorously. The casework samples analysed in this study revealed that 33% of the total 88 samples had foreign DNA matching a suspect [61]. The discrepancies between the lower foreign DNA detection of 30–40% of samples in [73] to 95% of samples in [157] may be due to differences in methodology. These differences include female participants being directed to scratch the forearms of male participants with both hands until red in the study conducted by Iuvaro et al. [75]. Their samples were also processed by using an AmpFlSTR® Yfiler® PCR Amplification Kit (Applied Biosystems by ThermoFisher, Foster City, CA, USA). This differs in comparison to the study conducted by Matte et al. [61], who had participants lightly scratch the forearms of their partner 10 times in one group and 30 times vigorously in another group [61]. This study utilised an AmpFlSTR™ Profiler System (Applied Biosystems) for amplification, highlighting another difference between the studies [61].

The utility of the fingernail samples in cases of sexual assault was investigated by Flanagan and McAlister [74], who assessed the transfer and persistence of DNA underneath fingernails after the digital penetration of either the vagina or anus of female victims. In the experimental part of the study, male participants, in a consenting relationship, performed vaginal digital penetration of their female partner, using each finger from the right hand. Samples were collected from under each fingernail of both the left (background) and right hands (post-activity) of the male participants [74]. The female partner's DNA was found in 31% and 94% of the left- and right-hand samples, respectively. The female donor was detected as the major contributor in all samples from the right hand, leaving the male undetected in all but one of the right-hand samples. This contrasts with the left-hand samples, where female DNA was detected as a minor component in all samples. Furthermore, four of the participating couples also submitted further samples at either 6, 12, or 18 h following digital penetration. The full DNA profiles of female participants were observed in 75% of samples at both 6 and 12 h, which decreased to 62% at 18 h. Further,

between 6 and 18 h, 25% of participants went from displaying only female DNA to revealing only the male donor's profile [74]. A retrospective study conducted by Nurit et al. [120] assessed the evidentiary value of 137 fingernail swabs recovered from a suspect or victim of homicide where there is suspected physical contact made during an offence [120]. Their analysis found mixed DNA profiles in 13.5% and 25% of suspect and victim fingernail samples, respectively. The researchers stated that the collection of DNA from underneath the fingernails of a victim can help investigators to determine suspected contact between a victim and offender, thus highlighting the value of this type of evidence.

8. Breast/Chest, Abdomen, and Back

8.1. Breast/Chest, Abdomen, and Back: Prevalence of Background DNA

Currently, there is no research examining the prevalence of background DNA found on the external skin of an individual's chest, abdomen and back. Investigations into background levels of DNA typically present on the skin of the breast, chest, abdomen and back would be valuable to forensic casework where these areas may have been contacted during offences involving physical contact such as kicking, shoving, groping/grabbing or actions common to sexual assault, where skin-to-skin contact may occur.

8.2. Breast/Chest, Abdomen, and Back: DNA Transfer and Persistence After Activity

In their retrospective study of 1499 sexual assault cases, Fonneløp et al. [38] assessed both autosomal and Y-chromosomal STR data from body samples collected from victims and suspects. A suspect's DNA was detected in 41% of the 73 breast and chest swabs collected for up to 43 h following the alleged offence [38]. Ramos et al. [76] demonstrated that DNA can be transferred from an offender to a victim's bra and underpants after contact over the top or underneath these garments during contact made to the breasts or chest area during a mock groping assault [76]. Higher DNA amounts were transferred to the external cups, the hood/clasp, and the underwire of the brassiere, highlighting the areas most likely to have DNA transferred following contact. However, the authors also stated that the extent of DNA transfer was dependent on the shedder ability of the offender making contact. The transfer of DNA from direct contact with the skin of the abdomen and back has not been explored. However, various papers have investigated direct DNA transfer from skin to clothing, including t-shirts [21,64,108,188].

9. Penis

9.1. Penis: Prevalence of Background DNA

Penile samples are commonly taken in cases of sexual assault [39]. If a DNA profile is generated, then questions from courts or investigators will often turn to the meaning of the results in the context of the case. To address these questions, it is essential to understand the types of DNA profiles that may be expected based on the proposed activities.

Taylor et al. [64] assessed the likelihood of detecting DNA and various biological fluids on a penis in the absence of sexual activity. In the study, 28 male participants were asked to refrain from sexual activity for 24 h, then wear previously prepared sterilised underwear for a full working day prior to submitting penile swabs and the underwear for analysis day [64]. Researchers conducted presumptive tests for blood, semen, and saliva, as well as screening the samples for the presence of hairs, fibres, and spermatozoa using microscopy [64]. None of the samples processed revealed the presence of blood and approximately 30% of the swabs indicated the presence of saliva on the penis. However, it should be noted that there is a potential for false positives results from various other trace materials to occur when testing for the presence of saliva using either of the Phadebas® or RSID™ Saliva test kits included in this study [189–191]. There were slightly higher rates

of semen detection, with 21% of the samples testing positive for acid phosphatase and approximately 33% recovering spermatozoa [64]. Furthermore, limited amounts of non-self DNA were recovered from the penile swabs, with 6 (21%) participant samples revealing between one and five non-self-alleles. These studies suggest the low likelihood of detecting non-self DNA from genetic material transferred to a penis following everyday activities.

9.2. Penile Samples: DNA Transfer and Persistence After Activity

The type of activity which might result in non-self DNA transfer to the penis includes vaginal, anal, and oral penetration; hand masturbation by another individual; and both direct or indirect contact by other individuals or objects. Fellatio reportedly occurs in 34% of male–male and 78% of male–female sexual assaults [192]. Dawnay et al.'s [86] review of the current bioanalytical methods adopted in forensic casework of sexual assault offences in the United Kingdom showed that 25% of penile samples were positive for the presence of amylase, found in saliva, and thus can be useful in cases where oral sex is alleged. Analogously, Keating and Higgs [80] found that 25% of penile swabs collected from sexual assault offences, then analysed with Phadebas® testing, were positive for saliva. However, there are limited data on the prevalence and detection of saliva on a penis in the absence of sexual activity. Taylor et al. [161] found that approximately 25% of samples analysed revealed the presence of saliva in the absence of sexual contact. The similarities in the frequency of detecting saliva on penises in cases of sexual contact and no sexual activity, as presented in each of these studies, suggests that the transfer of saliva can occur from a range of activities and may not be specific to sexual intercourse. Thus, further exploration of the frequency of saliva being detected in both instances should be considered, to allow for the accurate interpretation of a positive saliva result when analysing penile swabs in forensic casework. The potential occurrence of false positives should also be considered when analysing these results, as this could influence the accuracy of findings. Hausmann et al. [86] found vaginal cells in 50% of penile swabs up to 5 days following sexual intercourse by using Lugol's iodine staining. Keating [79] also evaluated the evidential value of 660 penile swabs collected from the forensic casework of sexual assault. The penile swabs were examined for the presence of at least one of the following biological substances: saliva, blood, faeces, vaginal secretions, and semen [79]. Their results indicate the following frequencies of detecting different biological materials: 9% were positive for saliva; 12% were positive for blood; 0.03% were positive for faeces (however, only 66 samples were analysed for the presence of faeces where faeces were suspected); 11% were positive for vaginal secretions; 47% were positive in an acid phosphatase test; and 13% detected spermatozoa. The overall findings of this research indicate the possibility of the detection of biological materials on penile swabs, and thus present value for establishing what likely activities occurred to result in the transfer of these materials to the penis. More recent studies investigated potential methodologies for the identification of vaginal secretions, and other biological materials, involving mRNA profiling techniques [84,187,193–196]. Building upon research that suggests the successful use of mRNA markers for the identification of biological stains [195] and vaginal secretions [197], Hadzic et al. [84] assessed the reliability of the MUC4 mRNA marker for identifying the presence of vaginal secretions on penile swabs in sexual assault cases. Their work found MUC4 mRNA markers in 79% of vaginal and 0.05% of saliva samples analysed [84].

Efforts have been concentrated on establishing the quality and quantity of female DNA that could be deposited on a penis following sexual intercourse [38,81,83,85]. Dawnay et al. [86] also stated that allegations of penile–vaginal penetration can be supported by the presence of vaginal fluid and epithelial cells from the vagina. This is supported by previous findings presented by Fonneløp et al. [38], who reported the detec-

tion of vaginal cells for 38% of penile samples obtained in sexual assault investigations. Kaarstad et al. [83] investigated the success rates of detecting female DNA from penile swabs or imprints collected during sexual assault through the detection of Lugol-positive cells. In the study, female DNA was detected on the surface of a penis in 49% of the cases taken on average 7 h following the assault (range of 1–15 h) [83]. Analogously, Cina et al. [81] investigated DNA transfer to a penis between one couple during sexual intercourse, finding that 22 ng of DNA was recovered from the penis 1 h after sexual intercourse involving penile–vaginal penetration, compared to 2.1 ng of DNA detected after 24 h [81]. Drobic [82] presented a case review highlighting the efficacy of penile swabs as a source of vaginal cells following a sexual assault. The DNA profile developed from the amplification of 15 STR loci from the penile swabs revealed the donor as the major and the female victim as the minor contributor to the sample [82].

Farmen et al. [85] analysed a series of post-coital penile swabs collected within 24 h of three consenting couples participating in sexual intercourse involving vaginal penetration. All samples collected following intercourse revealed female DNA on the penis, whereby the female participants' full DNA profiles were recovered in 90% of samples obtained between 5 and 12 h [85]. Across the penile samples, there was 100% frequency of recovering female alleles at 0 h, which gradually decreased to the lowest recorded frequency of 30% at 20 h. However, three couples revealed 100% of female DNA in samples collected 20, 22, and 24 h following intercourse. Male allele recovery ranged from 65% to 100% in samples, showing no trend or increase in male DNA recovery as time progressed, and the amounts of female DNA decreased. This contrasts with the 13% frequency of female DNA detection following non-intimate everyday contact seen in the study produced by Jones et al. [63]. Jones et al. [63] investigated the possibility of DNA being deposited on a penis via non-intimate contact, where male participants contacted females over the face, neck, and hands for 5 min and then simulated going to the bathroom to urinate. The quantity and frequency of female DNA detected on a penis and underwear from indirect contact were assessed by collecting worn male underwear and penile swabs at various time intervals following both non-intimate social contact and sexual intercourse [63]. Female DNA was detected in 13% of the penile samples collected immediately after the social interaction, and in none of the samples taken six hour later. In contrast, this same study found that 100% of samples obtained from the underwear and penis after sexual intercourse revealed female DNA [63]. The results of these studies not only underscore the utility of both presumptive biological tests and the DNA profiling of penile samples in cases of sexual assault, but also demonstrate that the outcomes of these tests and analyses may vary depending on the specific activities involved. This variability can aid in activity-level evaluations.

10. Vaginal Samples

10.1. Labia and Vulva: Prevalence of Background DNA

The labia, vulva, and mons pubis can be sampled following an alleged sexual assault to assess if any DNA was transferred by the perpetrator. However, understanding the mechanisms of DNA transfer from criminal activity requires an understanding of the background levels of DNA present on the target area. Murphy et al. [198] explored the prevalence of male DNA on the underwear of women, through the Y-STR analysis of 103 samples. This research found that only five samples had sufficient male DNA to develop a full Y-STR profile, which matched cohabiting male partners of female participants. This was notable given that over 80% of the female participants were cohabiting with a male. The researchers suggest that further exploration of background DNA on women's underwear as well as the external vagina is needed [198].

Breathnach et al. [65] investigated background levels of saliva and foreign DNA detected in labial and vaginal samples obtained from women who were asked to refrain from oral intercourse. Of the vaginal swabs collected from the labia, which were analysed for the presence of saliva (via Phadebas®, RSID™- Saliva immunological testing and mRNA profiling), 15.8% of the samples produced positive results from the 19 female participants [65]. Furthermore, 10.5% of the saliva-positive samples also revealed foreign DNA, possibly from indirect transfer to the tested areas [65]. However, further research should be conducted to establish the background levels of other biological materials and the prevalence of male DNA on the external vagina in the absence of criminal activity.

10.2. Labia and Vulva: DNA Transfer and Persistence After Activity

Most of the research conducted on the transfer of DNA and its persistence in vaginal samples focusses on internal samples. However, the DNA transfer to, and persistence on, external vaginal areas is also of interest, as it is common for DNA to be deposited to such areas following contact made in sexual assault offences. Willot and Allard [94] looked at the propensity for spermatozoa to persist by analysing 567 casework samples, finding that 61% of the external vaginal samples did not detect any spermatozoa. There was a marked drop in detection after 6 h, and the longest reported persistence of spermatozoa without tails was 120 h after deposition. Astrup et al. [95] also confirmed the ability to recover spermatozoa from the external genitalia of the vagina from samples collected within 24 h of sexual intercourse. After penile–vaginal penetration, 88% of samples saw the detection of spermatozoa. Furthermore, 14% of these samples were recovered from participants who reported no ejaculation, implying spermatozoa can still be transferred without ejaculation. This is due to discharge draining from the vagina to the labia, causing the displacement of spermatozoa to the labia, making information regarding if ejaculation did or did not occur relevant to sampling both areas [95]. Significantly better spermatozoa recovery was observed in the vaginal cavity and cervix compared to the labia and vulva. It should be noted that the time following intercourse was not published, limiting this aspect of the results.

10.3. Vagina and Cervix: Prevalence of Background DNA

Data conveying the likelihood of detecting different biological materials from vaginal and cervical samples in the absence and presence of sexual intercourse involving vaginal penetration are important for the assessment of different activities proposed [66,77,78,92,94,199]. Albani et al. [66] investigated if male DNA could be detected in the vagina of women following everyday activities, collecting 300 swabs from eight participants. Participating women were asked to collect a series of vaginal swabs across specific time periods and record their everyday activities, including if they had participated in sexual intercourse [66]. The quantification and Y-STR amplification of male DNA were assessed in this study [66]. Male DNA was detected during quantification in 21% of the samples, ranging between 0.10 pg/µL and 12.2 ng/µL in 50 µL of DNA extract [86]. None of the samples with a negative male quantification result obtained more than three Y-STR alleles, while 49% of samples with positive male quantification detected three or more alleles, all after reported intercourse. The authors also reported "unexpected" alleles, referring to alleles that either did not match the known male reference (in samples taken post-intercourse) or were detected in the absence of reported intercourse in 14% of the samples. In such profiles, the unexpected alleles were limited to three or fewer and 86% of these profiles were associated with the second category (donor did not report intimate contact). These findings suggest that the chance of male DNA detection in the vaginal cavity following everyday contact is remote, but more data are needed [66].

10.4. Vagina and Cervix: DNA Transfer and Persistence After Activity

Vaginal and cervical samples can be obtained by forensic or medical examiners, from both live and deceased victims where sexual assault is alleged [39,65,77]. The analysis of vaginal swabs generally consists of presumptive and confirmatory tests for saliva and semen, and DNA analysis [39,66,94,96,200]. Various methods of gynaecological sample collection—swabbing methods, cervical scrapings, and cervical brush sampling—have been investigated to optimise the recovery of DNA following a sexual assault where vaginal contact or penetration is alleged [79,95,201]. Gregory [99] presented an investigation of the transfer of biological materials, excluding spermatozoa, into the vaginal cavities of women following digital penetration, where saliva is and is not used as a possible lubricant. Their findings establish the possibility to detect sufficient male-derived DNA from the vaginal cavity of a female both 24 and 72 h following digital penetration. Across all samples, full profiles from the male were detected 19% of the time, and partial profiles were recovered in 78% of instances, with a total of 3% of samples revealing no male DNA. Their findings revealed no trend in the DNA amounts or quality of the profiles dependent on if saliva was used.

Loeve et al. [202] explored the implications of trace contaminations into the vaginal cavity during sample collection using a speculum, suggesting the use of a sleeve as a preventative measure. The use of a speculum sleeve during swabbing procedures was found to reduce false positives due to trace contamination from 87% to 2% [202]. Thus, this research highlights the importance of taking caution when sampling, to prevent contamination and preserve DNA evidence collected from the vaginal cavity.

The persistence of spermatozoa in the vaginas of women can be influenced by various factors, with survivability decreasing over time [77,92,94,98]. There are various studies dating back to the 1970s that investigated the persistence of spermatozoa in the vagina [38,89–93,95,203–206]. A summary of this research and the findings on spermatozoa persistence is presented in Table 2. Willott and Allard [94] investigated the detection of spermatozoa on vaginal swabs obtained during sexual assault offences and found that spermatozoa can be detected on the internal and external areas of the vagina after a number of days (summarised in Table 2) [94]. Magalhaes et al. [96] highlighted the age of the victim and the time following intercourse as key factors in the detection of spermatozoa, with detection of spermatozoa in the cervix possible up to 5 days following ejaculation [207]. Allery et al. [197] found that the maximum persistence of spermatozoa within a vagina is approximately 3 days [95].

Table 2. Summary of studies investigating the persistence and detection of spermatozoa from vaginal samples obtained following penile–vaginal penetration where ejaculation has occurred.

Author	Publication Year	Sample Size	Sample Type	Maximum Time of Persistence	Optimum Recovery Time Post-Intercourse	Recovery Rate of Spermatozoa at Max. Time of Persistence
Sharpe [88]	1963	Not indicated	Vaginal swabs and cervical smears	Motile sperm—6 h Non-motile sperm—up to 4 days	6 to 12 h	Not indicated
Morrison [89]	1972	178	Vaginal swabs and cervical scrapings	9 days in the vagina, 12 days in the cervix	48 h	58.4%
Leppaluoto [90]	1974	300	Cervical scrapings	7 days	<2 days	46%
Eungprabhanth [91]	1974	200	Vaginal swabs	7 days	48 h (80% recovery)	33% up to 5 days
Davies and Wilson [92]	1974	Not indicated	Vaginal swabs	6 days	<3 days	34% (between 90 and 156 h)

Table 2. *Cont.*

Author	Publication Year	Sample Size	Sample Type	Maximum Time of Persistence	Optimum Recovery Time Post-Intercourse	Recovery Rate of Spermatozoa at Max. Time of Persistence
Randall [206]	1987	349	Cervical scraping	7 days	<1 day (maximum 25% recovery rate)	14% (reordered at 5 days)
Silverman [93]	1978	675	Cervical smear	10 days	<1 day (approximately 65%)	25%
Ricci and Hoffman [204]	1982	90	Vaginal swabs	7 days	<1 day	Not indicated
Gould et al. [207]	1984	60	Vaginal swabs	5 days (motile sperm)	12 h	Not indicated
Allery et al. [197]	2001	174	Vaginal swabs	3 days	<72 h	35.1%
Culhane et al. [203]	2008	302	Vaginal secretions	Only investigates up to 2 days since intercourse	Not indicated	15.2%

Several studies highlight the high value of spermatozoa detection in sexual assault offences [77,200,208]. However, in sexual assault offences involving vaginal–penile penetration, ejaculation only occurs in approximately 50% of cases [38,39,97,209,210], making alternative biological source identification and analyses valuable following the collection of vaginal swabs. The use of Y-STR profiling has been particularly beneficial to sexual assault cases where there was no semen detected or where digital penetration may have occurred [72,97,210]. McDonald et al. [210] conducted a review of 47 cases of sexual assault where penile or digital vaginal penetration was alleged to have occurred and in the absence of spermatozoa. In the study, vulva and low vaginal swabs were analysed using Yfiler®, finding that a Y-STR profile with at least three alleles was detected in 30% of the cases, and at least ten alleles were detected in 21% of the cases [210].

Owers et al. [97] evaluated two Y-STR multiplexes for their ability to detect male DNA from vaginal swabs obtained during sexual assault cases where semen was not present. Yfiler® and PowerPlex®Y23 were used to amplify DNA from vaginal swabs from 250 sexual assault cases [97]. Usable profiles were generated from 17% and 34% of the cases using Yfiler® and PowerPlex®Y23, respectively [97]. These findings suggest PowerPlex®Y23 is more effective at producing Y-STR profiles compared to a Yfiler® kit [97]. Dziegelewki et al. [211] explored the ability to detect male epithelial cells, and Y chromosomes specifically, in the vaginal tract using fluorescent in situ hybridization (FISH). This method proved to be effective in identifying male epithelial cells with intact Y chromosomes from vaginal samples collected up to 7 days post-coitus [211].

Autofluorescence and morphological flow cytometry methods have recently been investigated for their ability to separate complex mixtures of biological materials recovered following an assault [72,212,213]. Flow cytometry is a common technique used in chemistry and medicine [72,212–214]. Ingram et al. [72] conducted a study investigating the use of imaging flow cytometry to distinguish cells from mixtures composed of vaginal and epidermal cells through autofluorescent and morphological characteristics. In the study, IFC methods classified epithelial cells with approximately 90% accuracy, suggesting potential usefulness in casework. This study, among other recent studies, exemplifies a growing area of research that provides potential protocols for epithelial cell type identification [72,186,212–215].

11. Buttocks/Anus

11.1. Buttocks/Anus: Background Levels of DNA

There is a scarcity of research exploring the prevalence of background DNA on the buttocks of individuals from everyday activities. In their study of background DNA levels detectable on the skin of children, Graham et al. [44] collected 30 samples from the right buttock and 29 from the left buttock of children, and found it is possible to recover non-donor DNA from these sites. While low numbers of non-donor alleles were detected (mean of 0.34 alleles from the left and 0.40 alleles from the right buttock), these findings suggest the ability to detect background levels of non-self DNA from the buttocks of children, but more data on both children and adults are needed. This would provide contextual value to the analysis of evidence from this site following an offence where contact is made to the skin of the buttocks [216].

Little is known about the presence of background DNA and its prevalence on anal, perianal, and rectal areas, and the samples collected from them. The collection of these samples can present unique challenges for the detection of DNA. Factors such as rapid DNA removal during defecation, the presence of bacteria that accelerate DNA degradation, and the potential for PCR inhibitors within the rectum can complicate analysis [217,218]. Magnetic bead extraction proved to be most effective at separating DNA from faecal matter, yielding between 40 and 136 ng of nuclear DNA, though recovered DNA proved too degraded to achieve successful STR profiling [62]. In a later study conducted by Vandenberg and van Oorschot [219], a QIAamp DNA Stool Mini Kit (Qiagen) was found to be effective at extracting sufficient DNA from 50 to 500 mg samples of different regions of faeces, and smears of faeces on cotton, for the generation of full DNA profiles using an AmpFlSTR Profiler Plus Amplification Kit (Applied Biosystems). Recently, the ability to detect DNA from the rectal cavities of rats for the purpose of genotyping was assessed, detecting 1800 bp long fragments of DNA suitable for PCR amplification [218]. The expansion of this area of research to address human samples could allow for a better understanding of expected DNA amounts in the absence of sexual contact with the anal cavity of humans.

11.2. Buttocks/Anus: DNA Transfer and Persistence After Activity

There is limited research exploring the transfer and persistence of DNA on the buttocks, despite this being a site where skin-to-skin contact might occur during a sexual assault offence. In their retrospective study, Fonneløp et al. [38] assessed 12 samples obtained from the buttocks and 17 from the rectums of victims, revealing DNA of interest from epithelial cells in 8% and 0% of the cases, respectively. Further, the study also assessed the persistence of spermatozoa, reporting detection in 19% of rectal swabs up to 24 h and 5% of rectal swabs up to 35 h following an alleged assault [38].

Allard et al. [77] also investigated the persistence of spermatozoa in the rectum at different times since intercourse. Of the 95 internal anal samples analysed, 4% detected spermatozoa up to 24 h post-deposition [77]. This research has been helpful in guiding more recent investigations into the persistence of spermatozoa in samples obtained following a sexual assault.

12. Legs, Ankles, and Feet

12.1. Legs, Ankles, and Feet: Prevalence of Background DNA

There is also an absence of data on the prevalence and persistence of DNA on an adult's legs. Graham et al. [44] obtained samples from the thighs and ankles of children aged 0–5 to assess the presence of any non-self DNA detectable in the absence of criminal contact. Their findings established that non-self DNA was present in approximately 0.4–1% of samples collected. These samples produced mixed DNA profiles with children as major

contributors and foreign DNA detected as minor partial contributors [44]. It should be noted that this research did not investigate background DNA on the feet or under the toenails of children or the prevalence of DNA on these body parts in adults, highlighting a gap in the current research.

12.2. Legs, Ankles, and Feet: DNA Transfer and Persistence from Activity

During an offence where physical assault occurs, contact to the legs of a victim (either live or deceased) may occur [37]. This presents the opportunity for DNA to transfer between the skin of an offender and that of the victim. Fonneløp et al. [38] found that in five samples obtained from the legs of sexual assault victims, 40% revealed the DNA of a person of interest presumably transferred via contact made during the offence. Van den Berge et al. [220] investigated the propensity for DNA to transfer to the knees and ankles of trousers during a staged offence scenario where the victim is dragged by the perpetrator. Samples were collected by tape lifting before and after dragging, to establish differences between background and post-offence DNA samples. A comparison of background and post-dragging samples revealed a 71% increase in the amount of DNA, with an average of 91% of grabber's DNA recovered. Although this can provide insights into DNA transfer during assault scenarios involving the grabbing of ankles, there has been limited research exploring the likelihood of DNA transfer directly to the skin of an individual's legs following various actions that may occur during such incidents. Research should focus on each specific body site to generate data that reflect the unique characteristics of each location. For instance, the skin on an individual's sole of their feet may be coarser and come into contact with different surfaces, with different histories and in different manners, than the skin of their thighs, affecting the likelihood of DNA transfer and persistence to these surfaces.

Similarly, there is limited research addressing the transfer of DNA and its persistence underneath toenails. The research conducted on the transfer of DNA under fingernails could be applied to this area [58,74,75,117–121]; however, future research specifically addressing DNA transfer and persistence on toenails is needed. It should also be noted that there is available research investigating the use of toenails to produce an STR profile identifying human remains [221,222]; however, this will not be addressed as this review focusses on DNA-TPPR.

13. Conclusions and Future Directions

There has been some progress in producing data to inform activity-level evaluations of DNA evidence recovered from body samples following contact during an offence. This has been achieved through the assessment of body samples collected and analysed under controlled conditions, and the evaluation of samples collected throughout forensic casework, in an attempt to establish its value. Many challenges still remain regarding DNA-TPPR of various biological materials on different external and internal body sites. The research reviewed here highlights efforts made to enhance our knowledge regarding body samples and identifies avenues for future research, including the following:

- Generating more data on the background levels of DNA specific to external skin surfaces and internal body sites commonly sampled in forensic casework where suspected contact is made (i.e., skin of the face, neck, chest/breast, penis, vagina, and hands);
- Establishing background levels of DNA on external skin and internal body areas less commonly sampled in forensic casework (i.e., the skin of the abdomen, back, arms, buttocks, anus, rectal cavity and perianal areas, legs, ankles, and feet);

- Determining who are the likely contributors of non-self DNA on different areas of a body relevant to different situations and activities, and where possible also the biological source(s) of this/these non-self component(s);
- Investigating the transfer and persistence of different biological materials (e.g., blood, semen, saliva, skin, earwax, and tears) to different common (e.g., breasts and faces) and uncommon (e.g., abdomen, back, and feet) body surfaces following different actions (e.g., kissing, biting, licking, grabbing, and pushing);
- Investigating the transfer and persistence of DNA to head hair following grabbing actions reflective of actions commonly seen in assault scenarios;
- Investigations of effects of various variables such as wearing and changing clothes, sharing towels, living with others, sharing a bed, washing clothes, etc., on the persistence of DNA transferred to the body;
- Further investigations into various recovery methods for sampling different body areas with the aim of maximising the yield of targeted non-self-DNA, and minimising the yield of non-targeted self DNA, recovered following a transfer event.

The key directions for future research on DNA-TPPR presented in this review are just a starting point. Numerous limitations in the existing research data highlight the need for further exploration in these areas to enhance our current understanding.

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