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Primary and Secondary Transfer of Touch DNA on a Knife: A Quantitative Analysis

By

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THESIS

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in

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Abstract

DNA is widely used as a reliable source of evidence in many criminal investigations. Touch DNA comes from samples deposited by hands and may not be a visible biological sample. This DNA can be transferred onto objects without direct contact. Real-life scenarios make it difficult to discern how DNA was deposited because of unknown activities prior. Individuals shed varying amounts of DNA when encountering other people or objects, therefore making it difficult to rely on a standardized source of DNA. Connecting these issues in forensic science, there is a need for reliable data compiled from known activities including standardized variables in the laboratory setting. This study establishes a series of pathways using known activities to investigate the potential quantity of DNA being transferred. Each pathway is identified as a direct or indirect transfer of touch DNA and replicated twenty times. A series of standardized variables for sample collection are used with a laboratory-created domesticated fingerprint acting as a positive control. Recovered DNA from each pathway is quantified, profiled, and identified as either a major or minor contributor. Results identified the last subject touching the end surface, a knife handle, as the major contributor of DNA quantity or profile. This data is useful in describing DNA transfer under these conditions and offer insight into the behavior of touch DNA in controlled environments relating to a common crime scene.

1. Introduction

Touch DNA has been a topic of interest in forensic science since the publication of “DNA Fingerprints from Fingerprints” by Roland A. H. van Oorschot and Maxwell K. Jones in 1997. They discovered that DNA is present on objects touched or held by someone (Oorschot and Jones 1997). This proved that DNA evidence does not have to be in the form of blood, saliva, or semen stains visible to the human eye. Locard’s Exchange Principle asserts when two objects come into contact, they leave a trace (Richard Saferstein qtd. in Mistek et al. 2019). The addition of Locard’s ideology to the known presence of touch DNA on human hands creates an opportunity to study the variables affecting transfer.

The foundation of touch DNA relies on cells on the human hand, palm, and fingertips that can then transfer to an object. Julia Burrill et al. identified components of touch DNA, in 2019, as five types of cells: endogenous nucleated cells, anucleate corneocytes, transferred exogenous nucleated cells, cell-free DNA, and fragment associated residual DNA (Burrill et al. 12). Keratinized cells lingering on the outer layer of skin from the hand are joined with nucleated epithelial cells that come from hands touching other parts of the body, or body fluids found near the mouth and nose. This establishes the human hand as a reliable source of DNA and broadens the spectrum of items for analysis.

Advancements in touch DNA techniques create a reliable environment analyzing small amounts of touch DNA. The conception of PCR (polymerase chain reaction) generated amplification of specific segments of DNA, using denaturation, annealing, and extension. This can detect low levels of DNA by creating millions of copies of DNA. Short tandem repeats were used with amplification to build a profile from DNA, targeting sections known as loci with a multi-step process of collection, extraction, and quantification before amplifying and generating

a genetic profile, there is potential for DNA loss at each step. The amount of DNA recovered can vary based on chosen methods of collection, analysis, and varying factors between individuals and objects.

The quantity of transferred touch DNA can vary between people and surfaces. Individuals varying in sex, age, and their inherent ability to leave behind DNA can change the amount deposited. Objects with differing surface types, such as porous or non-porous, change DNA deposition too. Analysis techniques like collection and extraction also contribute to recovery. Current research finds common variables that influence touch DNA deposition, transfer, and recovery that can be used to compare to samples recovered in casework.

1.1 Variability of Objects

The object receiving a transfer of touch DNA from someone can differ in many ways including porosity, duration of contact, and pressure. One approach to narrow these differences is to focus on the most common surface types encountered in crime scenes. Researchers classify surface types as porous or non-porous. Salem Alketbi and W. Goodwin focused on six different surfaces during their 2019 study: stainless steel, glass, and plastic as non-porous, and wood, banana skin, and copier paper as porous options. Larger quantities of DNA were recovered from the non-porous glass, while the porous paper option recovered the lowest. Cotton swabs were beneficial on non-porous surfaces, while tape was better for collection on porous surfaces (Alketbi and Goodwin). This suggests non-porous surfaces retain less DNA and allow more to be recovered, and it is important to specify collection method for surface type. This is supported in a 2019 study by Sophie Hartless et al., using glass, wood, and plastic as surface types. They identified a correlation between the number of alleles recovered depending on material and method of collection for different surface types. Wooden surfaces had more alleles recovered

when collected with a foam swab, while plastic surfaces had more when using a polyester swab (Hartless et al. 2019). The studies only had one collection method in common: cotton swabs. Although both studies chose similar surface types, analytical methods varied, making it difficult to identify different surface types as the sole factor contributing to DNA recovery. Both studies used different durations of contact which also led to different recovery rates. Alketbi and Goodwin had participants apply force to surfaces for one minute (Alketbi and Goodwin 704). Whereas Hartless et al. analyzed surfaces after participants held an object for five minutes, rotating it in their hands, repeatedly over seven days (Hartless et al. 379). Even though each study may use the same surface to compare data, differences in methodology can affect the impact of results. Duration can vary between studies, but pressure will directly influence the amount of DNA deposited.

Increased friction and pressure increase the amount of touch DNA transferred onto a surface. In 2017, Samuel Tobias et al. built on the studies of pressure between two objects but focused on data to support direct contact from hands (Goray et al. qtd. in Tobias et al. e12). They directly deposited fingerprints onto a polycarbonate board with varying amounts of pressure. While this study agreed with previous results, it also discovered pressure at low amounts did not show differences between individuals while higher pressures did, proposing pressure as an independent variable (Tobias et al. 2017). Since pressure increases the amount of DNA deposited, it can be a potential new method compared to the use of shedder status in studies.

1.2 Variability of Individuals

Individuals leave DNA on objects at differing levels. This is referred to as their shedder status and used as a variable corresponding to touch DNA transfer. In 2002, Alex Lowe et al. studied the idea that individuals shed differing amounts of DNA and compared profiles between

people after washing their hands. They clarified the difference between a good and poor shedder was the ability to generate a full profile from their deposits after washing their hands. A poor shedder needed six hours to regenerate after handwashing, while a good shedder could produce a full profile immediately after (Lowe et al. 25).

DNA deposition varies largely in real-life scenarios due to this shedder status.

Abdulrahman Oleiwi et al. studied six different individuals and quantified the DNA left from fingerprints on a glass slide. Their study showed recovery ranged from 0.00 – 1.56 ng of DNA between six individuals. The amount of DNA also varied based on collection location. More DNA was recovered from the fingertips than the palm, and in one individual the fingertips produced 0.359 – 1.56 ng while the palm only produced 0.00 – 0.229 ng. They also standardized handwashing to be an hour before with no intermediate contacts, which may have decreased recovery too (Oleiwi et al. 2015). Shedder status is highly variable and can affect how much DNA is deposited at a crime scene, but it is also useful in a laboratory setting to study indirect transfer of touch DNA onto objects.

1.3 Indirect Transfer

Originally in 1997, Ladd et al. concluded no secondary DNA transferred between individuals onto objects after conducting a study with varying times of handshakes (Ladd et al. qtd. in Cale et al. 196). In 2016, Cynthia Cale et al. disproved this theory citing the increased sensitivity of analysis instruments, and their own study involving a two-minute handshake between individuals varying in shedder status. They found 85% of their samples showed secondary DNA transfer (Cale et al. 196), and in 25% of samples a good shedder profile was the major contributor when their DNA was indirectly transferred by a poor shedder (Cale et al. qtd. in Szkuta et al. 2017). The presence of touch DNA being the result of indirect transfer is a

potential method of deposition on evidentiary items. This can be used to study other variables affecting the transfer of DNA.

Since two people with varying shedder status can pair for a handshake, there is also the possibility of standardizing the period of collection after interaction. Bianca Szkuta et al. studied the effects of activities happening after the handshake. They found DNA to persist up to fifteen minutes on a hand after a handshake and transfer indirectly even after routine activities take place. They also noted doing more activities after the handshake did decrease the amount of DNA collected. However, they found no correlation between handwashing and DNA deposition amounts in 24 participants, and they did not require handwashing before the handshake (Szkuta et al. 2017). Although unpredictable in real-life scenarios, shedder status can assist in the laboratory setting and help standardize other variables impacting transfer, prevalence, persistence, and recovery of DNA, commonly known as TPPR.

1.4 Transfer, Prevalence, Persistence, and Recovery

It has been established that DNA can transfer from individuals to objects according to Locard's Exchange Principle. Touch DNA can be transferred through a primary, direct, route to an object or it can be transferred through a secondary, indirect, route. Van Oorschot et al. describe DNA-TPPR in depth in 2019, focusing on factors affecting DNA transfer and the series of transfer events influencing the chances of recovering DNA. Increased sensitivity of instruments allows for decreasing quantities of DNA to be analyzed including mixture profiles. It is just as important to discover how the DNA was deposited, as to who it belongs to.

Van Oorschot et al. defines touch DNA transfer as the path DNA took before deposition. Direct transfer is described as DNA being deposited onto an object by direct contact. Indirect transfer covers a broad scope of potential transfer events after the initial deposition. However,

secondary transfer specifies a single transfer after the primary deposit (van Oorschot et al. 2019). For example, person A shakes hands with person B, then person B touches an object. The DNA from person A deposited on the object, if any, would be secondarily transferred. Unknown events can happen in between transfers and make reconstructing presumptions difficult. Quantifying DNA at each stage of the transfer process – before, during, and after – is crucial in a laboratory setting to establish baseline measurements of DNA presence across various objects and conditions.

DNA prevalence is what biological sources were deposited on objects prior to criminal activity. Maarten van den Berge et al. studied the prevalence of DNA over 549 samples supporting the idea that background DNA is found on public and private objects. The hand samples in their study showed donor alleles were higher than non-donor, but the number of contributors ranged between one and four, with 88% of hand samples containing background alleles (van den Berge 2016). Even though a high amount of DNA is recovered, it does not correlate to the number of contributors. Although prevalence, like shedder status, is difficult to predict in real-life scenarios, understanding it is valuable for eliminating background DNA in laboratory settings and ensuring accurate quantification.

Persistence of touch DNA is also affected by multiple factors including type of contact, duration, pressure, and shedder status. However, the environment largely determines DNA persistence. Jennifer Raymond et al. recognized variability of recovering touch DNA and studied effects of different environments on recovery process. They utilized a control sample kept inside, and when compared to outdoor samples they found the quantity of DNA decreased by half after two weeks and disappeared completely after six weeks. However, samples kept inside the lab were able to produce a full profile after six weeks even after losing half of the quantity

(Raymond et al. 2009). Surface type, environment, and time affects how long DNA can remain viable. Contacts made with other objects during activities before deposition decrease sample quantity but increase if repetitive contacts are made (Szkuta et al. 2017). Understanding the influential factors of touch DNA persistence is valuable in casework, guiding sample collection priority and supporting accurate profile interpretation.

Quantity of DNA recovered is dependent on the method used for collection and extraction. Timothy Verdon et al. studied nine different surfaces and found each one impacted the efficiency of extraction even when using the same method throughout the experiment. They emphasized the need for a correction factor, by comparing samples against a positive control for the Chelex™ extraction method used across different substrates (Verdon et al. 2013). The recovered amount of DNA can be increased by using specific extraction methods for different surfaces, or an adjusted calculation for the method is sufficient in calculating the original amount of DNA deposited. Standardizing influential variables increases DNA recovery, a useful tool in real-life casework, and helpful in distinguishing a series of events leading to DNA deposition, otherwise known as activity-level propositions.

1.5 Activity-Level Proposition

DNA is established to be transferable, persistent, prevalent, and recoverable depending on specific environments. Amounts of touch DNA recovered can help explain the mode of transfer and deposition to recreate a series of events. Based on evidentiary material, DNA deposition is widely accepted and the focus shifts to explain the series of events leading to its deposit. R. Cook et al., in 1998, describes the role of a scientist in the courtroom is to help hypothesize events that led to evidence being there. Evidence is organized by interpretation level in a hierarchy of propositions. This develops a framework that categorizes evidence based on different levels of

specificity and how it is evaluated by scientists, prosecution, or defense. It lets forensic scientists present and interpret evidence in a structured way based on the level of specificity. DNA persistence and transfer data help describe certain circumstances of how evidence was deposited and should be evaluated. The level in the hierarchy of propositions that relies on knowledge and interpretation from forensic scientists is known as the activity level (Cook et al. 1998). However, there are many variables affecting the recovery of DNA in real-life casework, and the sensitivity of testing can potentially generate profiles from persistent background DNA that is not criminally involved, making it challenging to discern a pathway of DNA transfer onto an object.

A proposition differs from an explanation due to the weight of the evidence. This is further emphasized by Ian Evett et al. in 2000, where they describe an explanation as a type of proposition not factoring in the weight of the evidence, such as the type of offense (Evett et al. 2000). Highlighted again in 2018, Duncan Taylor et al. points out DNA from contamination or specific transfer events is an explanation not applying to activity-level propositions. Instead, transfer should be used as a variable, including uncertainty, to calculate a likelihood of events before developing a proposition (Taylor et al. 2018).

In 2018, another study by Szkuta et al. analyzed the persistence of non-self-DNA on hands after a handshake, ultimately contacting a weapon after a set period. They noted the activities done in between by participants, objects contacted, and time delays developed a likelihood of DNA being directly or indirectly deposited. After 40 minutes and up to 5 hours after a handshake, they found indirect DNA was able to be transferred and detected on a surface. Although indirect transfer amounts were very low and decreased as the amount of time after the handshake increased (Szkuta et al. 2018). Studies using consistent variables establish specific events to help develop real-life case propositions.

1.6 Objectives

This study replicates DNA transfer pathways using standardized variables of collection method, extraction method, and surface type. To solve the variability of DNA from wild fingerprints, a laboratory-created domesticated fingerprint is created and acts as a known and standardized DNA positive control (Tang et al. 2020). The domesticated fingerprint is deposited onto a mock hand to enact different transfer scenarios. Transfer pathways include a primary, or direct transfer, and a secondary, or indirect transfer. DNA is tracked through different pathways calculating loss and retention percentages, bridging the gap between variation of real-life samples and laboratory methods of analysis.

A ground truth sample in the form of a reliable fingerprint containing a known quantity of DNA was developed by Jessica Tang et al. in 2020. They utilized human diploid cells suspended in inorganic solution to replicate a real touch DNA sample, counting the number of cells per microliter, and calculating the amount of cell suspension needed for 1500 cells, or nine nanograms (Tang et al. 2020). A positive control sample excludes variability of a wild fingerprint. A known starting quantity of cells allows accurate calculations of recovery when factoring in loss points of DNA during collection, extraction, and surface retention. Domesticated fingerprints are reproducible, and this study applied them to a mock hand, alongside wild fingerprints, investigating the effects of direct and secondary transfer of DNA on a knife handle after stabbing.

2. Materials and Methods

2.1 Decontamination

The knives (Surviveware 10.5-Inch Fixed Blade Tactical Knife with 6-inch Carbon Steel Blade and Rubber Wrapped Aluminum Handle for Camping, Hunting, EDC, and Survival) (https://www.amazon.com/Surviveware-10-5-Inch-Tactical-Aluminum-Survival/dp/B0CH8M8GS6/ref=sr_1_4?crd=FSVT7IADV2V0&dib=eyJ2IjojMSJ9.Gn5p6FAAVp3Xu83j0aEPWSQ0wX4A5ShFHhyxwaHlTdcfXP_r_19LfkIz7K6bwPe_98W2Og45I5Gh04u myMPZTFRghijAsWJ0ijFf4uaiRTmNaqHVP-qVayWcpmTdZc6tiekam_mRQjWVd4JvSQbeRApBz90IYx7o3M9eEUjQ2yp19HLUBjhupeW5f5E6ae7kLf93Z43zmkZxltXRYXbp8LQagi0dmvnkS2zpni1RGhAuZM_vaTzfrGQnpH-m6EDZzGR9c_OJVeDPGELb8_mfNMOG3JZsTbYhp2y6dDCkoQ.R4d5Iz6nYd_8crctOhGVgYncbOHHulNJ4hmdLyc5dJk&dib_tag=se&keywords=green+handle+surviveware+knife&qid=1755541057&s=sporting-goods&sprefix=green+handle+surviverware+knife%2Csporting%2C198&sr=1-4) and Lorica leather pieces (Ehrlich Leder, Biberach, Tübingen), fixed to Fisherbrand™ Extended Cuff Nitrile Gloves (Thermo Fisher Scientific, Waltham, MA), were decontaminated. This process included applying 100 µL of DNA Away™ (Thermo Fisher Scientific), 100 µL of 70% ethanol, and 100 µL of autoclaved water onto the surface of the knife handles or Lorica leather. Each reagent was allowed to dry, and Kimwipes™ (Kimberly-Clark Professional, Roswell, GA) were used to absorb any excess liquid. The surfaces were then placed in UV Stratalinker 1800 (Stratagene, San Diego, CA) for fifteen minutes under UV light.

2.2 Domesticated Fingerprint

Domesticated fingerprints consist of cell suspension, fingerprint solution (FS3), and 10X PBS. It must stay slightly agitated by gentle vortexing before pipetting onto leather pieces.

2.2.1 Cell Suspension

A cell suspension was created using a buccal swab from a donor with their informed consent under the approval of the Institutional Review Board at the University of California, Davis (Protocol 1956436). A sterile cotton-tipped swab (Puritan, Guilford, ME) was used to collect buccal cells. The swab was removed from the wooden shaft using a sterile scalpel (MedPride, Shield Line, Bayonne, NJ) and placed in a 1.5 mL microcentrifuge tube with 500 μ L of 1X Accumax™ Cell Dissociation Solution (Innovative Cell Technologies, Inc, San Diego, CA). The swab was incubated for fifteen minutes at room temperature and agitated by vortexing every five minutes for ten seconds. After fifteen minutes, the swab was removed and 500 μ L of Accumax™ was added before a second incubation for fifteen minutes, vortexing every five minutes for ten seconds.

2.2.2 LUNA™ Automated Cell Counter

A dilution of 1:1000 Diamond™ Nucleic Acid Dye (Promega, Madison, WI) was prepared by adding 99 μ L of molecular grade water to 1 μ L of 1:100 aliquoted Diamond™ Dye. Three aliquots containing cell suspension and Diamond™ Nucleic Acid Dye in a 1:1 ratio were dispersed into 1.5 mL microcentrifuge tubes respectively. Ten microliters of the 1:1 cell suspension and dye was dispersed onto three separate Luna™ Reusable Slides (Logos Biosystems, Annandale, VA) before being inserted into the LUNA-FL™ Dual Fluorescence Cell Counter (Logos Biosystems). A cell count was performed using Fluorescence cell counting

mode. Parameters for each count included: dilution factor: 2.00, minimum cell size: 5, maximum cell size: 60, and a red and green fluorescence threshold: 7. Each slide containing ten microliters was counted three times resulting in nine total cell count readings. The average of nine counts were used to calculate the concentration of the cell suspension (cell/ μ L).

2.2.3 Domesticated Fingerprint Calculations

The cell suspension concentration correlates to the volume needed to dispense nine nanograms of DNA. One epithelial cell is equal to six picograms of DNA and can be applied to the average cell count from the Luna™ Automated Cell Counter to calculate the volume of cell suspension needed (Tang et al. 2020). To calculate 1500 cells or nine nanograms the following equation is used:

$$1500 \text{ cells} \times \left(\frac{1 \mu\text{L}}{\text{average \# of cells}} \right) = \mu\text{L of cell suspension}$$

Each domesticated fingerprint solution had a final volume of 20 μ L containing: 2 μ L of 10X fingerprint solution (FS3), calculated volume of cell suspension, and 1X PBS as the remaining volume calculated by the following equation:

$$18 \mu\text{L} - \mu\text{L of cell suspension} = \mu\text{L of 1X PBS}$$

The sebaceous fingerprint solution (FS3) was made in two parts. Part one contained eccrine components in 100 mL sterile H₂O: 140 mg potassium chloride, 130 mg sodium chloride, 25 mg sodium bicarbonate, 17.5 mg ammonium hydroxide, 4.0 mg magnesium chloride, 27.5 g serine, 13.5 g glycine, 11 g ornithine, 8 g alanine, 4 g aspartic acid, 4 g threonine, 4 g histidine, 3 g valine, 3 g leucine, 190 g lactic acid, 50 g urea, 2 g pyruvic acid, 0.5 g acetic acid, and 0.5 g hexanoic acid.

The second part was combined in 20 mL of sterile water and contained sebaceous components: 50 mg hexanoic acid, 50 mg heptanoic acid, 50 mg octanoic acid, 50 mg nonanoic acid, 50 mg dodecanoic acid, 50 mg tridecanoic acid, 50 mg myristic acid, 50 mg pentadecanoic acid, 55 mg palmitic acid, 55 mg stearic acid, 50 mg arachidic acid, 55 mg linoleic acid, 55 mg oleic acid, 275 mg triolein (glyceryl triolate), 20 mg tricaprylin (trioctanoin), 20 mg tricaprin, 20 mg trilaurin, 20 mg trimystrin, 20 mg tripalmitin, 120 mg squalene, 30 mg cholesterol, 40 mg Cholesterol n-decanoate, and 155 mg cetyl palmitate.

The solution was made by adding sebaceous components to an amber bottle and mixing on a hot plate at 35°C using the magnetic stirrer. Equal amounts of the sebaceous mixture and the eccrine mixture were added to separate bottles. One milliliter of BRIJ S20 (0.05 mg/ml), made by dissolving 1 mg of BRIJ S20 in 20 mL of sterile water using a magnetic stirrer and hot plate at 35°C, was added to the bottles before mixing the final solution on a hot plate at 35°C with the magnetic stirrer. This solution cannot be autoclaved.

Chemicals were purchased from Fisher Scientific, Sigma Aldrich (St. Louis, MO) or Santa Cruz Biotechnology (Santa Cruz, CA).

2.3 Mock / Domesticated Hand

Lorica leather is a flexible material with similar consistency to real human skin. It is used as a mock skin that can be fixed onto a nitrile glove that, when worn, mimics the pressure and actions of a real human hand. Each piece of Lorica leather was cut into 1.5-inch and 1-inch squares before being glued onto nitrile gloves. The mock hands are gloves that have 1-inch by 1-inch squares fixed to the second fingertip. Domesticated hands are gloves that have 1.5-inch by 1.5-inch squares fixed to the palm area. When engaging in a handshake, the leather pieces come into direct contact with each other, and later the knife handle. The mock, or domesticated, hands

are worn by participants, and twenty microliters of the domesticated fingerprint is deposited onto the leather before performing a transfer pathway. In the primary domesticated pathway, the domesticated fingerprint was dispensed on the mock hand fingertip (Figure 2. A). In the secondary domesticated pathway, male DNA in the form of a domesticated fingerprint was dispensed onto the mock hand fingertip, and a female DNA domesticated fingerprint was deposited onto the domesticated hand palm (Figure 4. B).

2.4 Sample Collection

Wild fingerprints were deposited onto the knife handle in primary and secondary transfer. Participants had no limitations on activities but refrained from handwashing for at least one hour prior to collecting a sample. Wild pathway samples were collected from the index finger of male participants and from the palm of female participants (Table 1).

Sterile cotton-tipped swabs were moistened with 20 μ L of 2% SDS solution before surface sample collection, using the moistened side of the swab followed by the dry side (Thomasma and Foran 2013). Swabs were removed from the wooden shaft using sterile disposable scalpels. Tweezers were used to place the swab into a Spin-X™ Centrifuge Tube Insert (Corning, Corning, NY) in Costar™ Snap Cap Microcentrifuge Tubes (Corning) for organic extraction. Each pathway had a positive buccal control, negative extraction control, and a surface blank negative control. Surfaces of each mock hand, domesticated hand, and knife handle were decontaminated and sampled using sterile cotton-tipped swabs moistened with 20 μ L of 2% SDS solution to ensure no background DNA is present.

Table 1. Total number of samples collected from each surface throughout each pathway.

Pathway	Samples Collected				
	Wild Hand: Female Palm	Wild Hand: Male Fingertip	Mock Hand	Domesticated Hand	Knife Handle
Primary: Wild	N/A	N/A	N/A	N/A	1
Primary: Domesticated	N/A	N/A	1	N/A	1
Secondary: Wild	1	1	N/A	N/A	1
Secondary: Domesticated	N/A	N/A	1	1	1
Total Samples Collected = 9 x 20 replications = 180 samples					

2.5 Transfer Pathways

The chosen pathway recreated the action of stabbing using a knife, focusing on the primary and secondary transfer of DNA onto the knife handle. This was accomplished by participants directly holding a knife handle and stabbing or engaging in a handshake before holding the knife and stabbing (Tables 2 and 3).

Each pathway, primary and secondary, was replicated 20 times, using 20 μ L of a domesticated fingerprint applied to either a domesticated or mock hand. Each pathway was also replicated 20 times using wild fingerprints. All surfaces were swabbed using a cotton-tipped swab wetted with 20 μ L of 2% SDS. Samples were extracted using the phenol:chloroform:isoamyl alcohol organic extraction method and quantified using Quantifiler™ Trio DNA Quantification Kit (Thermo Fisher Scientific).

A. Primary Pathway

Table 2. Primary pathway schema with mock and wild hands.

<u>Primary Pathway</u>	
Wild Hand	Female hand → Stabs gel with knife
Mock Hand	(9 ng of Female DNA) Mock hand → Stabs gel with knife

A.1 Wild Fingerprint

A female participant refrained from handwashing for at least one hour prior. They held the knife handle, ensuring connection of the second finger, and stabbed the gel mold brain for ten seconds. Samples were collected from the knife handle where the second fingertip connected (Figure 1).

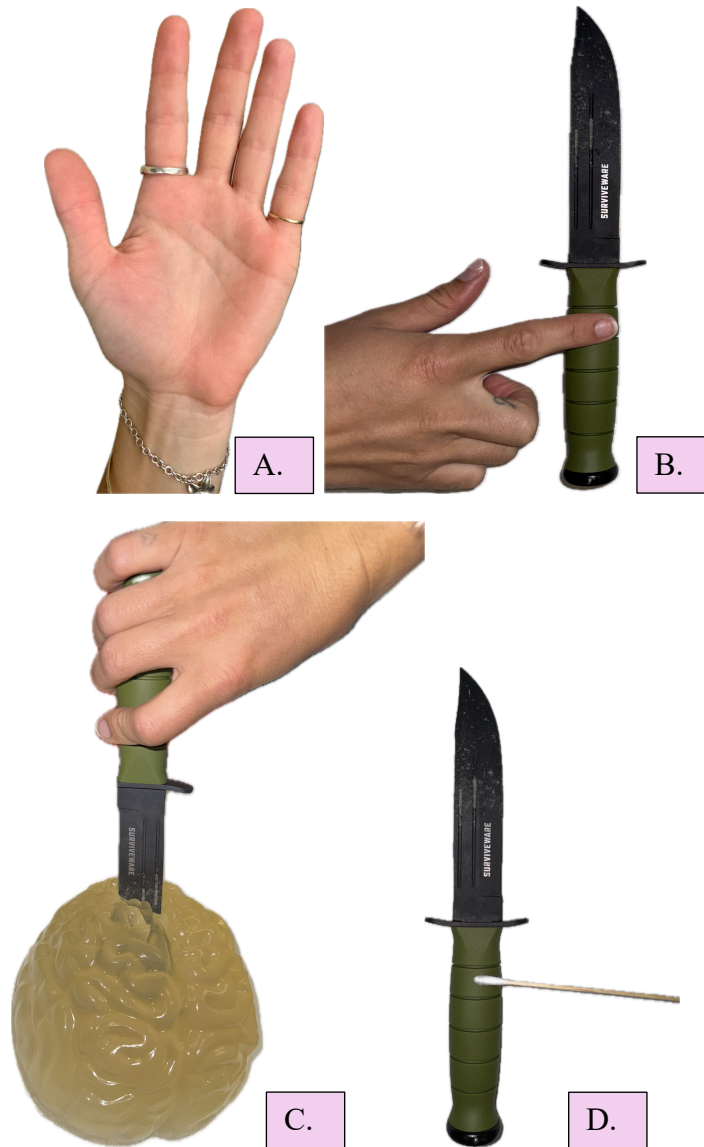


Figure 1. Visual schematic of a primary wild pathway. Female participant (A) places second fingertip on the second section of the knife handle (B). She holds the knife while stabbing the gel mold brain for ten seconds (C) and a sample is collected from the knife where second fingertip was located (D).

A.2 Domesticated Fingerprint

A mock hand with a 1-inch square of Lorica leather attached to the second finger was decontaminated to ensure no background DNA. Twenty microliters of a domesticated fingerprint, containing nine nanograms of female DNA, was deposited onto the leather piece.

The mock hand then held the knife with moderate pressure before stabbing a gel mold brain for ten seconds before releasing the knife. Samples were collected from the knife handle and leather piece containing the domesticated fingerprint (Figure 2).

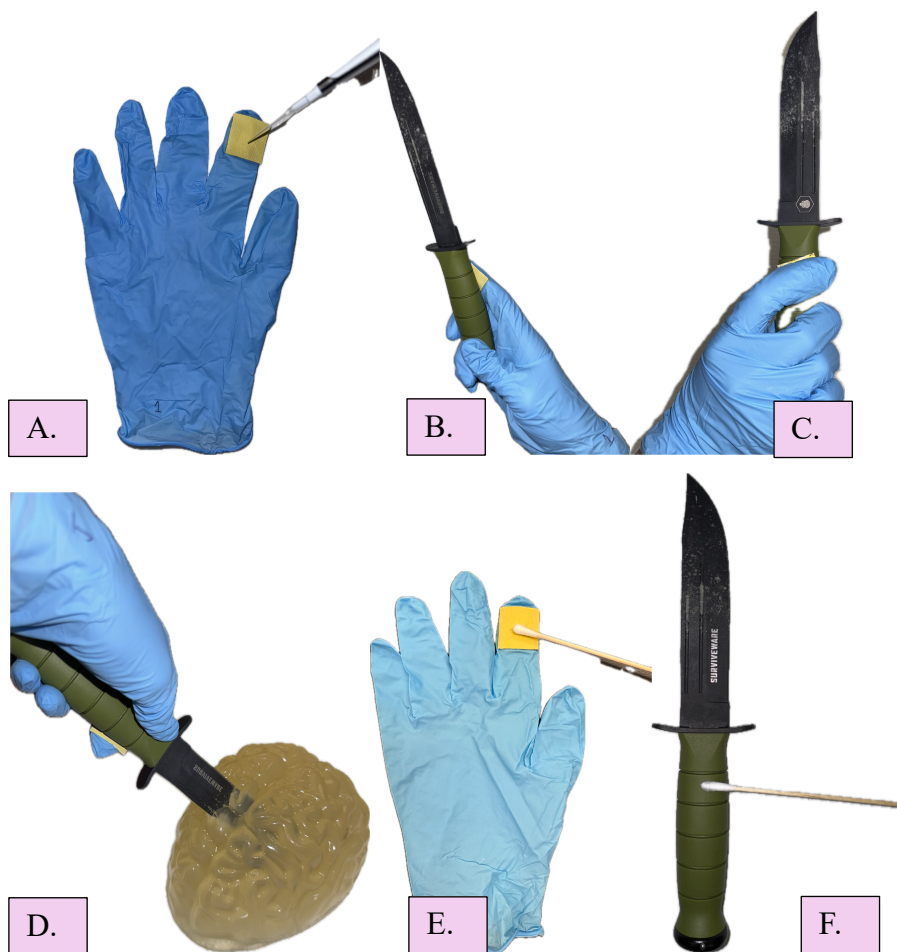


Figure 2. Visual schematic of a primary domesticated pathway. Nine nanograms of female DNA, in the form of a domesticated fingerprint, is deposited onto the mock hand (A) and then holds the second section of the knife handle (B) and (C). Then, the mock hand holding the knife stabs the gel mold brain for ten seconds (D). A sample is collected from the mock hand and the knife handle where second fingertip was located (E) and (F).

B. Secondary Pathway

Table 3. Secondary pathway schema with mock, domesticated, and wild hands.

<u>Secondary Pathway</u>	
Wild Hand	Female hand *shakes* Male hand → Male hand → Stabs gel with knife
Domesticated/Mock Hand	9 ng of Female DNA deposited on Domesticated hand (Female hand) *shakes* 9 ng of Male DNA deposited on Mock hand (Male hand) → Mock hand (Male hand) → Stabs gel with knife

B.1 Wild Fingerprint

Two participants, one male and one female, refrained from washing their hands at least one hour prior. The female participant shook hands with a male for ten seconds. Then, the male held the knife and stabbed a gel mold brain for ten seconds, ensuring connection of their second finger. Samples were collected from the female palm, male fingertip, and the knife handle (Figure 3).

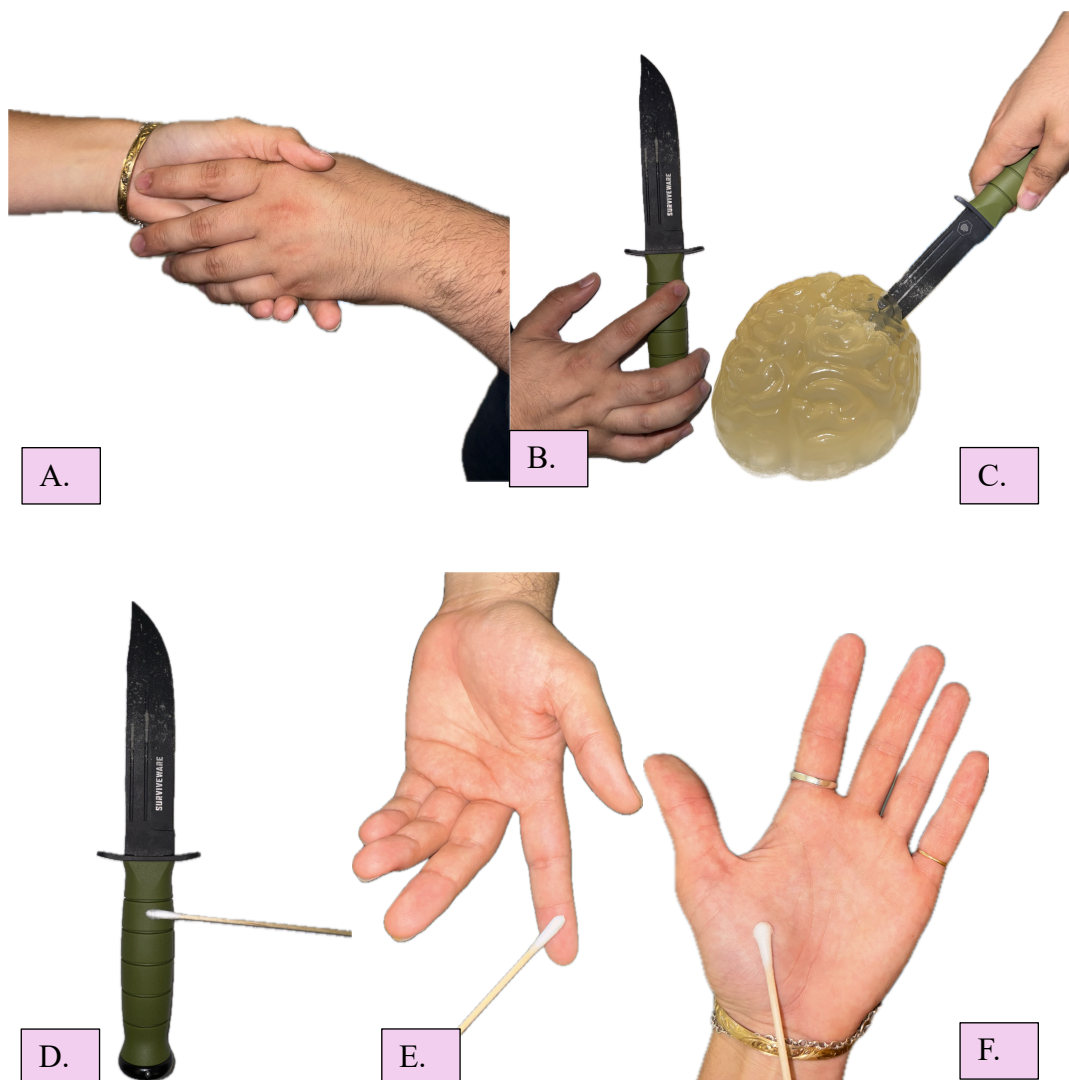


Figure 3. Visual schematic of a secondary wild pathway. The male and female participants shake hands for ten seconds (A) and then the male places his second fingertip on the knife handle (B) before stabbing the gel mold brain for ten seconds (C). The knife handle (D), male's second fingertip (E), and female's palm (F) are all swabbed for analysis.

B.2 Domesticated Fingerprint

A mock hand with a 1-inch square of Lorica leather fixed to the second finger received 20 μ L of a domesticated fingerprint containing nine nanograms of male DNA. Simultaneously, a domesticated hand with a 1.5-inch square of Lorica leather fixed to the palm received 20 μ L of a

domesticated fingerprint containing nine nanograms of female DNA. The male DNA mock hand shook hands with the female DNA domesticated hand, ensuring contact between the two domesticated fingerprints, for ten seconds. The mock hand then held the knife handle and stabbed a gel mold brain for ten seconds using moderate pressure. Samples were collected from the domesticated hand, mock hand, and knife handle after the pathway was complete (Figure 4).



Figure 4. Visual schematic of a secondary domesticated pathway. Nine nanograms of male DNA in a domesticated fingerprint is deposited on the mock hand (A) while nine nanograms of female DNA is deposited on the domesticated hand (B). Both hands shake for ten seconds ensuring contact between domesticated fingerprints (C). The mock hand contacts the knife handle on the

second section (D) before stabbing the gel mold brain for ten seconds (E). Each surface is swabbed for analysis including the knife handle (F), domesticated hand (G), and the mock hand (H).

2.7 Organic Extraction

Organic extraction using phenol:chloroform:isoamyl alcohol is efficient and yields high recovery of DNA when compared to automated methods (Ibrahim and Nassar 2018). This is essential when processing touch DNA samples and quantity is a high priority. Samples, collected using a sterile cotton swab, were cut from the wood shaft using a disposable sterile scalpel. After the addition of 400 μ L of Stain Extraction Buffer (1M TRIS-HCl pH 8.0, 5M NaCl, 20% SDS, 0.5M EDTA) and 15 μ L of Proteinase K (0.1 mg/mL) into each tube, samples were incubated for 12-18 hours in a 56°C-water bath. Swabs were placed into Spin-X™ Centrifuge Tube Inserts in Costar™ Snap Cap Microcentrifuge Tubes and centrifuged in Spectrafuge™ 24D Microcentrifuge (Labnet, Edison, NJ). To separate the phases, 400 μ L of phenol:chloroform:isoamyl alcohol (24:25:21, pH 8.05) was added to each sample, and the aqueous layer was removed into a 1.5 mL microcentrifuge tube. DNA precipitated in 1 mL of cold 100% (absolute) ethanol for at least 1 hour in -20°C. Samples were centrifuged for 5 minutes at 16,300 g to pellet DNA in each tube. Samples were then washed two times with 1 mL of 70% ethanol, dried for ten minutes during 56°C incubations in the LSE™ Mini Incubator (Corning), and re-solubilized with 30 μ L of molecular-grade water before incubating in a 56°C-water bath for 12-18 hours.

2.8 Quantitation

Decontamination controls and positive control samples were quantified using Alu-SYBR-Green-specific AB 7500 Real-Time PCR System (Nicklas and Buel 2003) (Applied Biosystems,

Waltham, MA) with HID Real-Time PCR Analysis Software. Eight standards were made from 200 ng/ μ L and 50 ng/ μ L stock DNA (Bio-Rad, Hercules, California) diluted to the following concentrations: 16.7 ng/ μ L, 5.56 ng/ μ L, 1.85 ng/ μ L, 0.62 ng/ μ L, 0.21 ng/ μ L, 0.068 ng/ μ L, 0.023 ng/ μ L, 0.0077 ng/ μ L. This created the standard curve. Ten microliter reaction volumes consisted of: 2 μ L of purified DNA, 0.4 μ L of forward and reverse primer each (forward: GTCAGGAGATCGAGACCATCCC, reverse: TCCTGCCTCAGCCTCCCAAG) (Sigma Aldrich, St. Louis, MO), 0.4 μ L of BSA, 5 μ L of 2X iTaq™ Universal SYBR® Green Supermix (Bio-Rad), and 1.8 μ L of sterile molecular-grade water (Cytiva, Marlborough, MA). A 96-well plate containing reaction samples was sealed and centrifuged before analysis. Cycling conditions were: 95°C for two minutes, 35 cycles of 95°C for fifteen seconds followed by 68°C for one minute. Generation of a melt curve ranging from 65°C to 95°C confirmed the single PCR product.

All transfer pathway samples were quantified using Quantifiler™ Trio DNA Quantification Kit and analyzed using HID Real-Time PCR Analysis Software (Thermo Fisher Scientific). The standard curve ranged from 0.005 ng/ μ L to 50 ng/ μ L with a total of five data points and was created using Quantifiler™ THP DNA Standard diluted with Quantifiler™ THP dilution buffer. Twenty-microliter reactions were prepared using: 2 μ L of extracted DNA sample, 8 μ L of Quantifiler™ Trio primer mix, and 10 μ L of Quantifiler™ THP DNA Standard diluted with Quantifiler™ THP dilution buffer. Four targets were identified for amplification: T.SA (small autosomal human genomic DNA), T.Y (genomic male DNA), T.LA (large autosomal human genomic DNA), and T.IPC (internal positive control). Manufacturer settings were followed for threshold, baseline, and dye settings. Cycling conditions were 95°C for two

minutes, 40 cycles of 95°C for nine seconds followed by 60°C for thirty seconds, generating a standard curve, melt curve, and amplification plot.

Female DNA was calculated using the following equation: $T.SA - T.Y$. Male DNA was quantified based solely on the T.Y target quantity. A degradation index was calculated from the following equation: $\frac{T.SA}{T.LA}$.

2.9 Amplification and Analysis

Purified DNA was amplified and profiled using PowerPlex Fusion® Amplification Kit (Promega, Madison, WI) according to the manufacturer's protocol. Reactions of 25 µL were prepared using: 1 µL purified DNA (0.5 ng/µL), 5 µL PowerPlex Fusion® 5X Primer Pair Mix, 5 µL PowerPlex Fusion® 5X Master Mix, and 14 µL amplification grade water. Samples were placed in GeneAmp® PCR System 9700 (Thermo Fisher Scientific) and cycling conditions were: 96°C for one minute, 30 cycles of 94°C for ten seconds, 59°C for one minute, 72°C for thirty seconds, 60°C for ten minutes, hold at 4°C. Amplified DNA was prepared for profiling in 11 µL reactions using: 1 µL of amplified DNA, 9.5 µL deionized formamide, and 0.5 µL WEN Internal Lane Standard 500. The 96-well plate was sealed with a Septa (Thermo Fisher Scientific) and cooled to 4°C in GeneAmp® PCR System 9700. All samples underwent capillary electrophoresis using SeqStudio™ Genetic Analyzer Instrument (Thermo Fisher Scientific) with the following parameters: Promega 5C Dye Set, Oven Temperature: 60, Pre-Run Voltage: 13,000 V, Pre-Run Time: 180 s, Injection Voltage: 1200 V, Injection Duration: 7 s, Run Voltage: 9000 V. Profiles were analyzed using GeneMapper™ ID-X Software (Thermo Fisher Scientific).

2.10 Retention Calculations

Potential loss of sample during analysis impacts the recovery methods of touch DNA samples. Extraction method, swab retention, and the surface are key fail points where significant quantities of DNA can be lost. Tang et al. (2020) calculated average DNA loss as a percentage. The average of the twenty samples recovered is the mean DNA recovery. The mean DNA loss is calculated by subtracting the mean DNA recovery from the total DNA deposited. Together this creates a percentage to calculate the average DNA loss: $\left(\frac{\text{mean DNA loss}}{\text{total DNA deposited}}\right) \times 100$.

Applying this calculation to standardized samples, they found DNA loss for Puritan® swab samples to average 25% and DNA loss due to organic extraction procedure using phenol:chloroform:isoamyl alcohol to average 34%. A combined average of 59% of DNA is lost due to swab and extraction. The percentage of DNA retained on the surface is calculated using the percentage loss of each element as follows: $\text{surface retention} = (\% \text{ loss surface}) - (\% \text{ loss swab})$, $\text{swab retention} = (\% \text{ loss swab}) - (\% \text{ loss extraction})$ (Tang et al. 2020). These calculations are represented in Table 4.

Table 4. Retention and loss percentage calculations using swab and extraction variables. The surface loss varies on the mean DNA recovered.

% Retained on surface	$(\% \text{ loss on surface}) - (\% \text{ retained on swab}) + (\% \text{ loss in extraction})$
% Loss on surface	$100\% - \left(\left(\frac{\text{mean DNA recovered}}{9 \text{ nanograms}}\right) \times 100\%\right)$
% Retained on swab	25% (Tang et al. 2020)
% Loss in extraction	34% (Tang et al. 2020)
Total % DNA loss	$(\% \text{ loss on surface}) - (\% \text{ loss on swab})$

The additional surfaces in this experiment were previously standardized. The knife handle has a retention of 10% and Lorica leather has a retention of 8% based on previous studies in UC Davis Forensic Science Center Laboratory under Dr. Ashley Hall.

2.11 Statistical Analysis

Pathways were separated into four categories: Primary Wild, Primary Domesticated, Secondary Wild, and Secondary Domesticated. All four pathways were compared using an analysis of variance (ANOVA) test to determine any statistically significant differences between the average recovery from the knife handles in each pathway. Each type of primary and secondary transfer was compared respectively using a *t*-test to confirm any differences between the pathways. Standard deviations were calculated for the knife handle samples in each pathway, but standard deviations for female DNA cannot be calculated due to the female DNA being a calculation itself ($T.SA - T.Y$).

3. Results and Discussion

3.1 Primary Pathways

A.1 Wild Fingerprint

The primary pathway was replicated twenty times using fingerprints from female volunteers, to represent real-life scenarios. Participants were asked to refrain from handwashing for at least one hour prior to deposition, although other activities were not disclosed outside of this parameter. The knife handle surface was decontaminated prior and contained no background DNA. Female participants directly held the knife while stabbing a gel mold brain for ten seconds, and samples were collected from the knife handle.

Results showed an average of 0.79 ng of female DNA and 0.02 ng of male DNA recovered from the knife handle. Female DNA ranged from 0.08 – 4.67 ng, supporting the notion of individual variation (Figure 5). Small amounts of male DNA were also recovered and could be attributed to activities in shared spaces prior to collection but after handwashing.

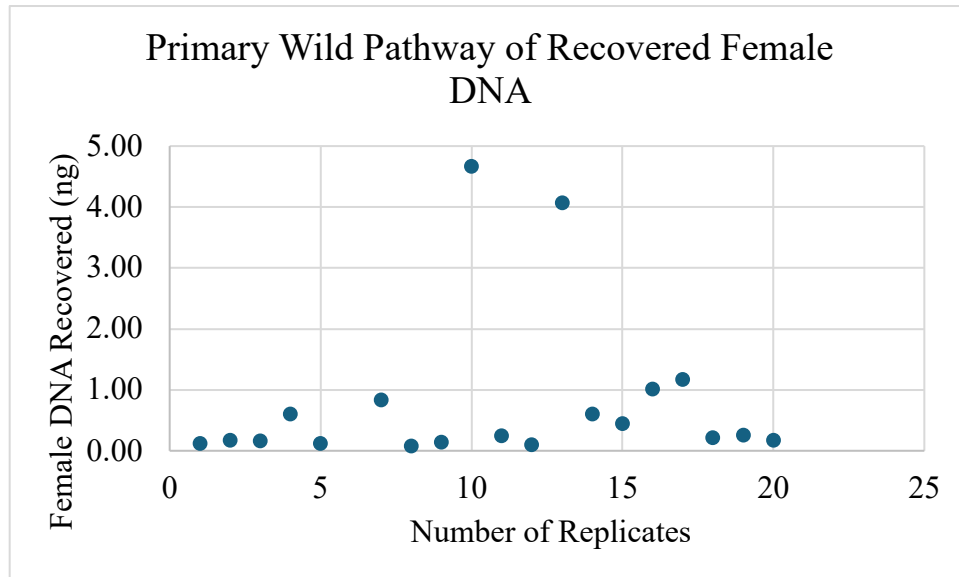


Figure 5. Scatter plot generated from the amount of female DNA recovered from the knife handle after a direct transfer. Values range from 0.08 – 4.67 ng, with an average of 0.79 ng. One replicate resulted in an undetermined amount and is not represented on the scatter plot.

The amount of female DNA recovered, when adjusting for loss, is 2.54 ng (Table 5) and calculations resulting in the adjusted recovery are shown in Table 6. The unadjusted amount in Table 5 describes the DNA that was quantified after the pathway took place. After applying the adjustment calculations to account for loss due to swab, extraction, and surface the adjusted recovery is listed below. In twenty replicates, over half of the average DNA was lost from the standard processing of a sample. The method of quantitation, Quantifiler Trio™, evaluates a degradation index (DI) by dividing the small autosomal (T.SA) by the large autosomal (T.LA). Results higher than one corresponds to degraded DNA. The degradation index ranged from 0.59 – 2.11 showing 9 out of the 20 samples had a DI over one indicating degradation (Table 7). Samples were collected immediately after deposition and were in a controlled environment of a laboratory setting, excluding environmental factors as a contribution. Low sample concentration could be attributed to the variation of individuals and their shedding status, and a small collection

area of the knife handle. These factors combined with standardized processing loss might explain a lower value with some samples having degradation.

Table 5. DNA recovered from wild female fingerprints involved in a direct transfer onto a knife handle. Recovery amounts are separated by male and female and show the adjusted DNA recovery based on loss calculations.

Unadjusted Recovery		
Surface	Female DNA (ng)	Male DNA (ng)
Knife Handle	0.79	0.02
Adjusted Recovery		
Surface	Female DNA (ng)	Male DNA (ng)
Knife Handle	2.54	0.05

Table 6. Percentages of total DNA recovery applied to the recovered female DNA in the primary wild pathway to adjust for loss.

Adjustment Calculations – Female DNA				
Raw Data	Adjustment Calculation	Data Adjusted for Loss (ng)	Total DNA Loss (%)	Total DNA Recovery (%)
0.79 ng knife handle recovered	$0.79 = x(0.31)$	2.54	69	31

Table 7. Degradation index calculated from female DNA recovered from knife handle. DI over 1 indicates degradation, showing 9/20 samples to be mildly degraded.

Replicate Number	Degradation Index
1	1.13633286
2	0.58832768
3	2.10769157
4	0.7840493
5	1.12996722
6	N/A
7	1.35918493
8	2.00376277
9	0.79021841
10	0.85451661
11	1.36713661
12	1.31881017
13	1.45385838
14	1.11226694
15	0.60741408
16	0.87210637
17	0.63547675
18	0.75061297
19	0.63390875
20	0.63351251

The total DNA recovered from the knife handle presents a standard deviation higher than the total mean (Table 8). This can be due to variance, which is expected between individuals, and the wide range of sample quantities compared to the average.

Table 8. Statistical parameters for the total DNA (ng) recovered from the knife handle after a direct transfer from female participants.

Knife Handle	Total DNA (ng)
Mean	0.81
Range	0.08 – 4.67
SD	1.30

Knife samples were subject to sample evaporation prior to profiling, and a select few were available to amplify and profile. Five primary wild samples are shown individually with each being compared to the reference female that deposited the sample. Allele numbers were replaced with an alphabetical system to protect identity. The amelogenin locus was not applied to this system and remains as X and Y alleles. Also, loci that did not recover alleles are titled 'N/A' and don't represent an allele site. Alleles that cannot be attributed to the female reference are identified in gray (Figure 6). Since the samples came from wild fingerprints and participants were asked to refrain from handwashing, gray allele sites are most likely background DNA on hands. Results show a female profile as the major contributor across all five samples, even with male DNA being quantified in low amounts on the knife handle. Results show that the major contributor of each recovered profile is from the last person touching the knife handle, directly transferring their DNA.

LOCI	FEMALE 1				FEMALE 2			FEMALE 3		
	ALLELE	KNIFE 1	KNIFE 2	KNIFE 3	ALLELE E	KNIFE 4		ALLELE	KNIFE 5	
Amel	X Y	X Y	X	X	X	X		X	X	
D3	P R	P R Q	P R	P R Q	O	O		P Q	P Q	
D1	O QQ	O QQ	O QQ	O QQ	L RR	L RR		P Q	P Q	
D2S441	K	N K V	K	P K	J N	J N		PP	PP	
D10	O	O	O	O	N	N		M O	M O	
D13	K M	K M	K M	K M L	K	K		H K	H K	
PentaE	N	N	N	N	J	N/A		K	K	

D16	Q	Q	Q	Q	R		L	L
	K	I K	K	K	I M	I M	K L	K L
D18	L	L	L	L	K M	K M	O R	O R
D2	R S	R S	R S	R S	P S	P	T Y	T Y
CSF	L	N/A	L	L	J L	J L	K L	K L
PentaD	I M	I	I M	I M	J L	L	I L	I L
THO1	I OO	I OO	I OO	I OO	F G	F G	G H	G H
vWA	R	R Q	R	R H	P	P	P Q	P Q
	CC	CC	CC	CC	DD EE	DD EE	CC DD	CC DD
D7	I K	K	I K	I K	J M	J M	H	N/A
D5	K M	K	K M	K M	K L	K	L	L
TPOX	H	N/A	H	H	H K	N/A	H	H
DYS391	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
D8	H J	H J I L	H J I	H J O	N	N	M N	M N I
	R Q	R	R	R	R	R	O R	O R
D19	N O	N O	N O	N O	M	M	M N	M N
FGA	W Y	W Y	W Y	W Y	T W	T W	V W	V W
D22		X				Y		
	O Q	O	O Q	O Q	O P		K P	K

Figure 6. Alleles corresponding to each knife sample recovered are compared against the reference sample. Allele numbers are replaced by letters. Each reference is to the left of the corresponding samples and color coded.

A.2 Domesticated Fingerprint

The domesticated fingerprint was prepared to contain nine nanograms of female DNA and dispensed on the mock hand leather square located on the second fingertip. The primary pathway involved the mock hand contacting the knife handle and stabbing a gel mold brain for ten seconds with moderate pressure. Prior to the pathway, all surfaces were decontaminated, showing the knife handle and mock hand to contain no background DNA. This pathway was replicated twenty times, and all replicates showed no degradation in the primary mock pathway.

The total amount of DNA recovered from the knife handle averaged 2.56 ng and the mock hand recovered 3.13 ng. Both surfaces had a slightly lower standard deviation than the mean (Table 9). The primary wild pathway had a standard deviation higher than the mean indicating a larger variance of samples collected, which is expected with wild fingerprints. In theory, using a domesticated fingerprint eliminates variance, and should result in a much lower standard deviation than the mean indicating consistent samples. In this pathway, the difference between standard deviation and mean is not large enough to state that variation was eliminated.

Table 9. Total DNA (ng) recovered from knife handles after a primary domesticated pathway depositing female DNA.

Total DNA (ng)	Mock Hand	Knife Handle
Mean	3.13	2.56
Range	0.39 – 9.03	0.64 – 6.21
SD	2.43	1.41

The total DNA recovery is adjusted to account for loss throughout the pathway. Key fail points, swab and extraction retention, are combined with the individual surface retention. Each fail point has a specific retention rate (Table 10). Lorica leather has a retention of 8%, when combined with swab (25%) and extraction (34%) loss the total DNA loss is 67%, and total DNA recovery becomes 33%. The female DNA recovered from the mock hand is divided by the total DNA recovery, 0.33, to result in an adjusted recovery of 8.73 ng (Table 11). Under the same method, male DNA recovered from the mock hand is 0.76 ng (Table 12). The knife handle has a retention of 10%, when combined with swab and extraction the total DNA loss is 69% and recovery is 31%. Adjusted female DNA recovered from the knife handle is 7.81 ng, and adjusted male DNA is 0.45 ng. Total female DNA recovered from the primary domesticated pathway, combining the mock hand and knife handle, is 16.54 ng or 183.78% calculated from:

$\left(\frac{\text{total DNA recovered}}{9 \text{ nanograms}}\right) \times 100$ (Table 11). Total male DNA recovered from the primary

domesticated pathway is 1.21 ng or 13.43% (Table 12). Cell suspensions were created from female buccal swabs, and participants were asked to not eat or drink one hour prior. Male DNA could be attributed to behaviors before donation.

Table 10. Comparison of retention percentages for each surface and controlled variables. Values are used to calculate the adjusted amount of DNA recovery after performing a pathway using a domesticated fingerprint.

Surface	% Retention
Knife Handle	10%
Mock/Domesticated Hand	
Lorica leather	8%
Loss Points	
Swab	25%
Organic Extraction	34%

Table 11. Recovered female DNA, after a direct transfer, adjusted for loss due to swab, extraction, and surface, to calculate a total of 16.54 nanograms.

Adjustment Calculations – Female DNA				
Raw Data	Adjustment Calculation	Data Adjusted for Loss (ng)	Total DNA Loss (%)	Total DNA Recovery (%)
2.88 ng mock hand recovered	$2.88 = x(0.33)$	8.73	67	33
2.42 ng knife handle recovered	$2.42 = x(0.31)$	7.81	69	31
	Total	16.54 ng	183.78 % DNA recovered	

Table 12. Recovered male DNA, after a primary pathway using a female domesticated fingerprint and adjusted for loss, totaling 1.21 nanograms from the mock hand and knife handle.

Adjustment Calculations – Male DNA				
Raw Data	Adjustment Calculation	Data Adjusted for Loss (ng)	Total DNA Loss (%)	Total DNA Recovery (%)
0.25 ng mock hand recovered	$0.25 = x(0.33)$	0.76	67	33
0.14 ng knife handle recovered	$0.14 = x(0.31)$	0.45	69	31
	Total	1.21 ng	13.44 % DNA Recovered	

The unadjusted amount of female DNA recovered from the mock hand averaged 2.88 ng, with a range of 0.39 – 8.78 ng. This amount of female DNA remained on the mock hand, while 2.42 ng was recovered from the knife handle. Although the domesticated fingerprint was created using female DNA, an average of 0.25 ng of male DNA was also recovered from a single mock hand sample (range 0.00 – 0.25 ng, SD 0.00 ng). The knife handle averaged a total of 2.56 ng recovered (2.42 female DNA and 0.14 ng male DNA) (Table 13). In total, more DNA was recovered from the mock hand than the knife handle, which could be due to collection area. The knife handle segment swabbed was smaller in size compared to the mock hand 1-inch square, and it also contained grooves in between each segment potentially trapping DNA from the domesticated fingerprint. Also, an improper number of cells were likely dispensed in the fingerprint due to clumping.

Table 13. Female and male DNA recovery from surfaces in a primary domesticated pathway. The adjusted recovery is calculated based on the swab, extraction, knife handle, and Lorica leather retention values respectively.

Unadjusted Recovery		
Surface	Female DNA (ng)	Male DNA (ng)
Mock Hand	2.88	0.25
Knife Handle	2.42	0.14
Adjusted Recovery		
Surface	Female DNA (ng)	Male DNA (ng)
Mock Hand	8.73	0.76
Knife Handle	7.81	0.45

Five samples from the primary domesticated pathway were profiled and compared against two reference profiles respectively (Figure 7). The samples compared to female 1 had more loci available to compare and matched between 22 – 40 alleles throughout all the knife samples. Samples compared to female 2 produced a lower number of loci and alleles, ranging from 10 – 16 alleles matching. This difference corresponds with sample quantity, as knives 4 and 5 recovered less than two nanograms, whereas knives 1, 2, and 3 all quantified higher than two nanograms. A low recovery amount could contribute to a larger partial profile. Two of the five samples produced profiles with no outside alleles, colored in gray, present. Since background DNA was eliminated on all surfaces, the most likely source of outside alleles comes from either the buccal swab when creating a cell suspension or low RFU values that are still above the threshold. All outside alleles had RFU values that ranged from 120 – 244 and the limit of detection threshold was set at 110 RFU. Although outside alleles are present, they exist in very low quantity, and each female reference is still the major contributor to the recovered sample. Based on profiles recovered from a direct domesticated pathway, a direct transfer of DNA onto a knife handle is expected to result in profiles that show one major contributor.

The profile of knife 1 is shown as an example of how alleles were assigned in Figure 8, where the female reference is identified in the allele boxes and the corresponding letter ‘F’ is placed next to the peak. Any alleles that were not identified above the limit of detection threshold or as an allele are marked with an asterisk next to the peak.

	FEMALE 1				FEMALE 2		
LOCI	ALLELE	KNIFE 1	KNIFE 2	KNIFE 3	ALLELE	KNIFE 4	KNIFE 5
Amel	X	X	X	X	X	X	X
D3	P Q	P Q	P Q O	P Q	O	O	O
D1	P Q	P Q	P Q	P Q	L RR	L	L
D2S441	PP	PP	PP	SS PP	J N	N	N/A
D10	M O	N/A	M O	M O	N	N/A	N/A
D13	H K	N/A	N/A	H O	K	N/A	N/A
PentaE	K L	K	N/A	K L	J R	N/A	N/A
D16	K L	K L	K L	K L	I M	I M	I M
D18	O R	N/A	O R	O R	K M	K	K M
D2	T Y	T	T	T Y	P S	N/A	N/A
CSF	K L	N/A	N/A	K L	J L	N/A	N/A
PentaD	I L	N/A	N/A	I L	J L	N/A	N/A
THO1	G H	G H	G H	G H	F G	F G	F G
vWA	P Q	P Q	P Q	P Q	P	P	P
D21	CC	N/A	CC	CC	DD	EE	N/A

D7	DD			DD	EE		
	H	N/A	H	H	J	J	N/A
D5	L	N/A	N/A	L	M		
					K	N/A	N/A
TPOX	H	N/A	N/A	H	L		
					H	N/A	N/A
DYS391	N/A	N/A	N/A	N/A	K		
					N/A	N/A	N/A
D8	M	M	M	M	N	N	N
			I	N			I
	N	N	N	I			
D12	O	O	O	K			
	R		R	R	R	R	R
D19			TT				
	M	M	M	M	M	M	M
	N	N	N	N			
FGA	V	V	V	V	T	N/A	N/A
	W	W	W	W	W		
D22				UU			
	K	K	N/A	VV	O	O	N/A
	P			K	P		
				P			

Figure 7. Alleles representing the knife handle samples collected after a primary domesticated pathway. Each reference is listed to the left of its respective samples and color coded.

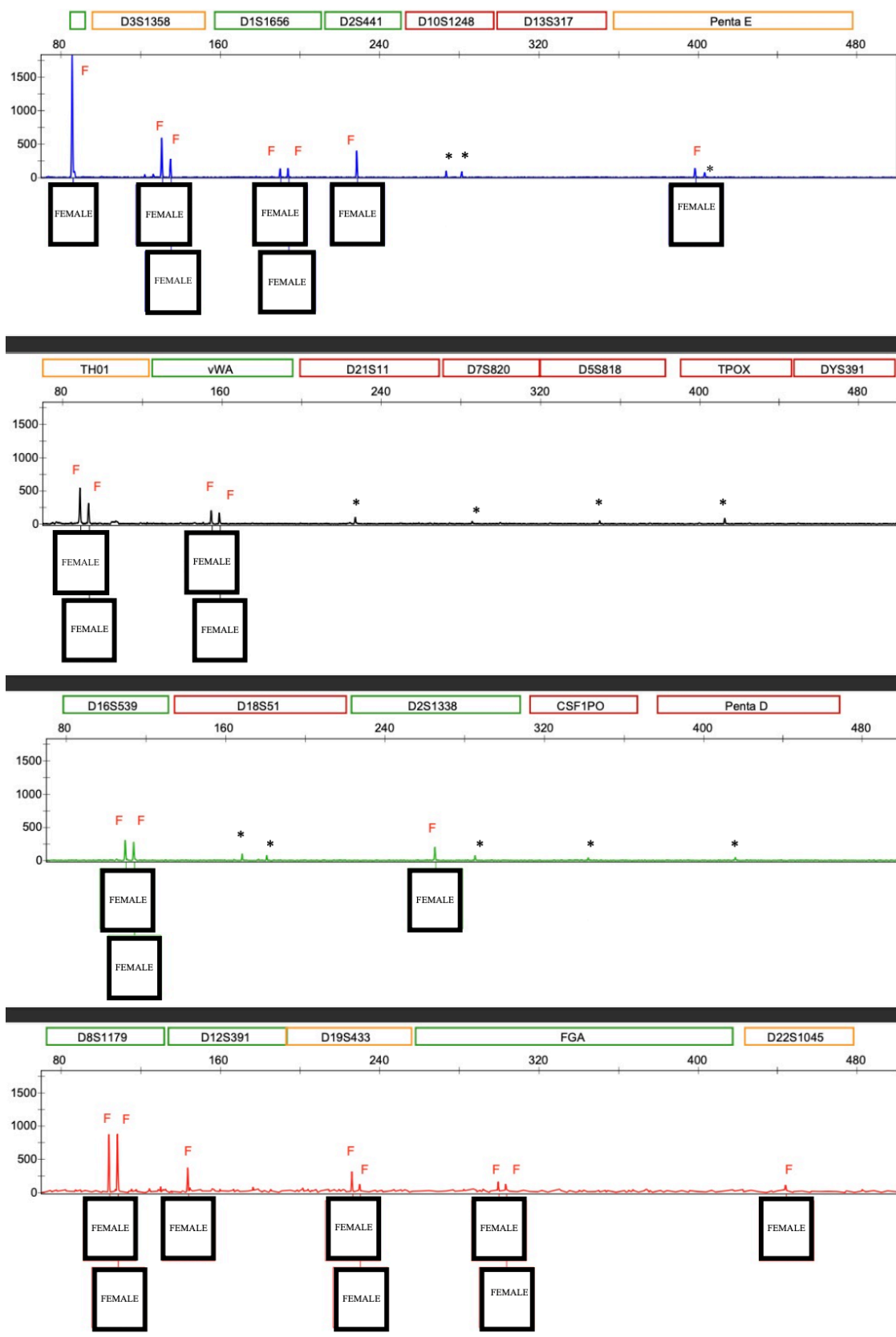


Figure 8. Profile recovered from a knife handle sample after a primary domesticated pathway with female DNA.

However, there is a notable gain of DNA when adjusting for loss. Surfaces were thoroughly decontaminated and resulted in undetectable amounts of background DNA before each pathway took place, eliminating contamination as a source of excess DNA. The likely source is an improper quantity of original DNA in the domesticated fingerprint. When creating a female cell suspension, the average cell counts could have been incorrect due to improper preparation. A high recovery indicates cell clumping, and when dispensing the fingerprint cell clumps include more cells than predicted. Although the amount of DNA recovered is higher than expected, the primary pathway supports the direct transfer of DNA from a single source when using a domesticated fingerprint. The use of a mock hand with a deposited domesticated fingerprint is sufficient in transferring DNA to the surface of a knife handle and shows the highest quantity recovered from the original source of DNA.

3.2 Secondary Pathways

B.1 Wild Fingerprint

The secondary pathway represented a study of indirect transfer of female DNA onto the knife handle. Two participants that refrained from handwashing for at least an hour shook hands for ten seconds. Immediately after, the male participant held the knife handle and stabbed a gel mold brain for ten seconds. Both female and male hands were swabbed, although the male hand collection was from the second fingertip and the female collection was from the palm area, like the placement of Lorica leather on the mock and domesticated hands. This pathway was repeated twenty times.

The secondary wild pathway involves direct and indirect transfer between individuals and surfaces. After the handshake, the male hand contained a large amount of male DNA, 5.43 ng, and a small amount of female DNA, 0.22 ng. In comparison, the female hand after the handshake had a lower amount of female DNA, 0.50 ng, than male DNA, 0.72 ng (Table 11). This indicates a direct transfer of DNA between the male and female hands after a handshake. It highlights individual variation and shedder status of each participant. The female and male hands were also swabbed in different locations, with the male collection being the second fingertip and female being the palm. In previous studies (Oleiwi et al. 2015) results show lower recovery from the palm area than the fingertips of individuals, which is recognized in the secondary wild pathway as well.

Table 11. Secondary wild pathway results of female and male DNA deposited on each hand and the knife handle after a handshake.

Surface	Female DNA (ng)	Male DNA (ng)
Female Hand	0.50	0.72
Male Hand	0.22	5.43
Knife Handle	0.00	0.83

The knife handle recovered an average of 0.83 nanograms of male DNA and 0.00 nanograms of female DNA. This shows the last person to touch the knife handle would be the higher contributor in quantified DNA recovery from the knife. Female DNA was recovered in low amounts indicating no indirect transfer in 10 out of 20 replicates and, in eight samples, indirect transfer had lower levels of female DNA than male DNA recovered, ranging from 0.02 – 0.17 ng (Figure 9). In two replicates (14 and 16) in Table 12 show slightly higher recoveries of female DNA than male DNA on the knife handle. A potential pairing of high shedder individuals, male, and low shedder females could explain the very low indirect recovery and the

variability between samples. This indicates the ability of an indirect transfer of DNA onto the knife handle after a handshake. Shedder status and collection location on each hand may have influenced the low deposition of female DNA directly and indirectly.

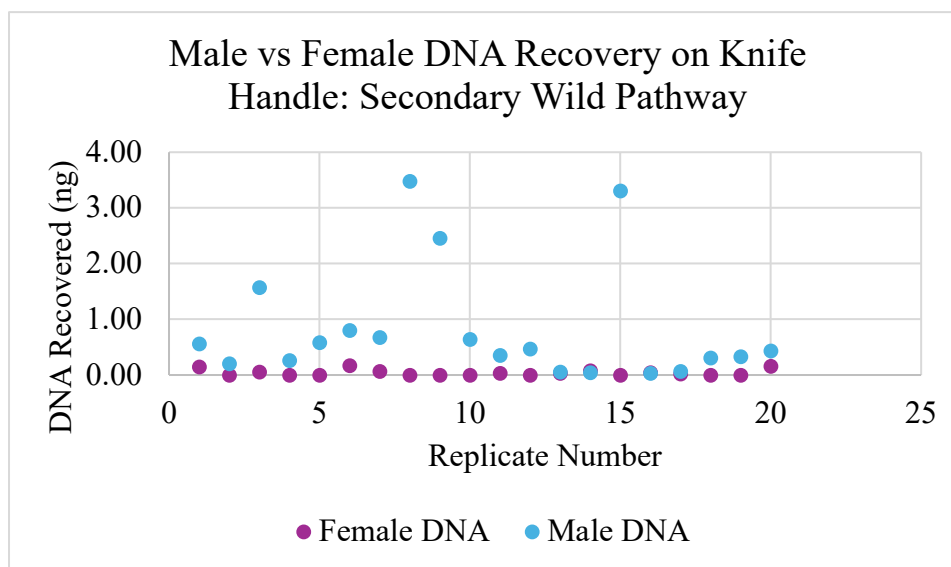


Figure 9. Female and male DNA (ng) recovered from knife handles after a secondary wild pathway. Each replicate is represented in the x-axis, showing ten of twenty recovering 0.00 ng of female DNA.

Table 12. Female and male DNA recovered from the knife handle after each replicate of the secondary pathway took place.

REPLICATE NUMBER	FEMALE DNA (NG)	MALE DNA (NG)
1	0.14	0.56
2	0.00	0.21
3	0.06	1.58
4	0.00	0.26
5	0.00	0.58
6	0.17	0.80
7	0.06	0.67
8	0.00	3.48
9	0.00	2.46
10	0.00	0.64
11	0.04	0.35
12	0.00	0.47
13	0.03	0.06
14	0.08	0.04
15	0.00	3.31
16	0.04	0.03
17	0.02	0.06
18	0.00	0.31
19	0.00	0.33
20	0.16	0.44

The adjusted recovery of DNA in the secondary wild pathway only applies to the male DNA since the average female DNA recovered is 0.00 ng (Table 13). Application of the calculations remain the same as previous and are shown in Table 14.

Table 13. Average recovery of female and male DNA from each surface after twenty secondary wild pathways as the unadjusted, or quantified, amount. Adjusted recovery of the knife handle is calculated for male and female DNA applying the loss percentage of swab, extraction, and knife handle surface.

Unadjusted Recovery		
Surface	Female DNA (ng)	Male DNA (ng)
Female Hand	0.50	0.72
Male Hand	0.22	5.43
Knife Handle	0.00	0.83
Adjusted Recovery		
Surface	Female DNA (ng)	Male DNA (ng)
Knife Handle	0.00	2.68

Table 14. Adjustment calculations of average male DNA recovered after twenty secondary wild pathways accounting for loss due to swab, extraction, and knife handle surface.

Adjustment Calculations – Male DNA				
Raw Data	Adjustment Calculation	Data Adjusted for Loss (ng)	Total DNA Loss (%)	Total DNA Recovery (%)
0.83 ng knife handle recovered	$0.83 = x(0.31)$	2.68	69	31
	Total	2.68 ng		

Standardized values of the total DNA recovered from the knife handle showed a higher value than the average (Table 15). Like the primary wild pathway results, it shows a pattern in wild fingerprint recovery. Due to the variation in individuals, it shows that recovery rates can differ by large amounts and influence the average recovery.

Table 15. Standardized results of knife handle samples after twenty replicates of a secondary wild pathway. The total DNA includes male and female DNA recovered in nanograms.

Knife Handle	Total DNA (ng)
Mean	0.77
Range	0.07 – 2.88
SD	0.87

Profiles of four knife handle samples were recovered after a male and female shook hands, and the male stabbed the gel mold brain holding the knife handle. Recovered profiles were compared to two reference profiles corresponding to each sample. In Figure 10 the alleles are color coded to the reference it most likely originated from, and alleles that are unmatched are coded in gray. Alleles that are shared between both the male and female references are coded in red. A profile could have many shared alleles and still identify a major contributor, but difficult to identify a single or mixed profile. For example, knife 1 has 36 alleles recovered and 13 of them are shared, but 21 are contributed from the male while only 1 is contributed from the female. Even with many shared alleles, it is still likely the male is the major contributor but not identified as a mixed profile. In knife 2, a total of 50 alleles were recovered (12 shared, 30 from male, 5 from female, and 3 from outside sources). This profile confirms a higher allele recovery of the person that directly touched the knife handle and lower allele recovery of the person indirectly transferred. It is likely a mixed profile of the two references which is expected after a secondary transfer. The profile recovered from knife 3 shows many outside alleles (19) present that do not match either reference. In comparison, the rest of the alleles contain 12 shared, 21 contributed from the male, and 11 contributed from the female. Higher outside alleles could be

from the lack of handwashing from both hands before deposition. This is a mixed profile, and the male would likely be the major contributor. Finally, knife 4 recovered a very partial profile that does not contain enough loci or alleles to conclude a mixed profile. Only containing 5 alleles, 1 is from an outside source, 1 is shared, and 3 are from the male reference. Reviewing four profiles, after performing a secondary pathway with wild fingerprints, it is expected to recover a mixture profile that includes shared alleles and identifies the male, or last person to touch the object, as the major contributor.

	MALE 1	FEMALE 1		MALE 1	FEMALE 2	
LOCI	ALLELE	ALLELE	KNIFE 1	ALLELE	ALLELE	KNIFE 2
Amel	X	X	X	X	X	X
	Y		Y	Y		Y
D3	YY	O	O	YY	O	O
	O			O	P	
D1		L	L		M	L
	RR	RR	RR	RR	Q	RR
D2S441	N	J	N	N	J	N
	O	N	O	O	K	O
D10	L	N	L	L	P	L
	P		P	P		P
D13	J	K	J	J	H	J
	K		K	K	L	K
PentaE	G	J	G	G	E	G
	L	R		L	K	L
D16	K	I	K	K	I	I
	L	M	L	L		K

D18	M	K	M	M	N	L
	N	M	N	N	P	M
D2	W	P	Z	W	T	T
	Z	S		Z	X	W
CSF	J	J	K	J	J	J
	K	L		K	K	K
PentaD	K	J	K	K	K	K
	L	L	L	L	M	L
THO1	F	F	F	F	I	F
	G	G	G	G	OO	G
vWA	Q	P	Q	Q	P	I
	R		R	R	R	Q
D21	BB	DD	BB	BB	BB	BB
	XX	EE		XX	CC	CC
D7	J	J	J	J	H	J
	L	M	L	L	J	L
D5	I	K	N/A	I	K	K
	K	L		K	M	
TPOX	H	H	N/A	H	I	H
		K			K	
DYS391	J	N/A	N/A	J	N/A	J
D8	M	N	M	M	J	J
	N		N	N	M	M
D12	P	R	P	P	Q	N
	ZZ		ZZ	ZZ	R	QQ

D19	L	M	L	L	N	L
	M		M	M	AC	M
FGA	W	T	W	W	T	U
	Z	W	Z	Z	X	W
D22	K	O	K	K	P	Z
		P				K

	MALE 1	FEMALE 3		MALE 2	FEMALE 4	
LOCI	ALLELE	ALLELE	KNIFE 3	ALLELE	ALLELE	KNIFE 4
Amel	X	X	X	X		N/A
	Y		Y	Y	Y (female)	
D3	YY	P	O	N	O	M
	O	Q	P	L	Q	N
D1	RR	P	N	O	K	O
			L			
			Q			
			RR			
D2S441	N	PP	AD	J	K	N/A
			ZZ			
			N			
D10	L	M	O	O	M	N/A
			L			
			O			
D13	J	H	P	K	L	N/A
			J			
			M			
PentaE	G	K	L	L	K	N/A
	L	L	M	Q	O	
D16	K	K	K	J	K	N/A
	L	L	L	M	L	
D18	M	O	M	Q	P	N/A

	N	R	N	R	Q	
			L			
D2	W	T	Z	Q	R	N/A
	Z	Y	V	V	W	
CSF	J	K	J	J	J	N/A
	K	L	K		K	
			L			
PentaD	K	I	I	M	M	N/A
	L	L		N		
THO1	F	G	F	F	OO	F
	G	H	G			
			H			
vWA	Q	P	P	P	N	N/A
	R	Q	Q	Q	R	
			R			
			S			
D21	BB	CC	BB	EE	DD	N/A
	XX	DD	CC	WW	WW	
			WW			
			XX			
D7	J	H	H	K	J	N/A
	L		J	L	K	
D5	I	L	I	K	K	N/A
	K		K	L		
			M			
TPOX	H	H	K	H	I	N/A
				L	K	
DYS391	J	N/A	N/A	J	N/A	N/A
D8	M	M	M	L	M	N/A
	N	N	N	N		
			I			
			J			
D12	P	O	P	S	R	S
	ZZ	R	ZZ	V	S	
			R			
			U			
D19	L	M	L	AB	M	N/A
	M	N	M	O	N	

FGA	W	V	N			
	Z	W	W	T	T	N/A
			T	U	X	
D22	K	K	O	P	L	N/A
		P			P	

Figure 10. Profiles generated from knife handle samples after a secondary wild pathway. Male and female references are to the left of the respective sample collected. Gray represents an outside allele, while red represents a shared allele between male and female.

B.2 Domesticated Fingerprint

A secondary pathway is established as an indirect transfer of DNA involving a male and female participant shaking hands and the male holding the knife handle. Two hands denoted mock hand as the male source and domesticated hand as the female source use a domesticated fingerprint created from a male and female participant. This pathway focuses on the female DNA that is indirectly transferred to the knife handle.

The mock hand, with deposited male DNA, and the domesticated hand, with female DNA deposited, shake hands for ten seconds before the mock hand holds the knife and stabs a gel brain mold for ten seconds. The surfaces of each hand and the knife handle are swabbed and analyzed. After the handshake, the female domesticated hand contained 0.71 ng of female DNA and 0.82 ng of male DNA, indicating more male DNA transferred onto the domesticated hand than female DNA onto the mock hand. The mock hand, after the handshake and holding the knife handle, contained 0.36 ng of male DNA and 0.19 ng of female DNA (Table 16). This result indicates more female DNA transferred from the mock hand onto the knife handle than the remaining amount on the mock hand.

The percent recovery of female DNA on each surface is determined by dividing the female DNA recovered by the total DNA recovered from the same surface and multiplying that

value by 100%. For example, female DNA was calculated to account for 46.3% of the total recovery on the domesticated hand: $\left(0.71 \text{ ng} / 1.54 \text{ ng}\right) \times 100\% = 46.3\%$. This recovery of female DNA may suggest more female DNA readily transferred to the male mock hand during the handshake, and therefore onto the knife handle resulting in the similar recovery rate from the end surface. The knife handle, only held by the mock hand after the handshake, recovered 0.27 ng of male DNA, or 52.18% of the total amount, and 0.24 ng of female DNA, or 47.49% (Table 16).

Table 16. Average unadjusted DNA recovery from each surface after twenty replicates of a secondary domesticated pathway. Percent recovery represents the amount of female or male DNA recovered from each surface type.

Female DNA		
Surface	Nanograms	Percent Recovery
Domesticated Hand	0.71	46.30%
Mock Hand	0.19	33.94%
Knife Handle	0.24	47.49%
Male DNA		
Surface	Nanograms	Percent Recovery
Domesticated Hand	0.82	53.50%
Mock Hand	0.36	65.52%
Knife Handle	0.27	52.18%

Adjustment calculations account for the swab, extraction, and two separate surfaces used in this pathway to produce adjusted recovery amounts (Table 17). An adjusted total result of 3.49 ng or 38.8% of female DNA was recovered from all surfaces (Table 18). In contrast male DNA, when adjusted for loss, resulted in a total of 4.44 ng or 49.3% of total DNA recovered (Table 19).

Table 17. Adjusted recovery amounts of female and male DNA from each surface throughout the secondary domesticated pathway.

Adjusted Recovery		
Surface	Female DNA (ng)	Male DNA (ng)
Domesticated Hand	2.15	2.48
Mock Hand	0.57	1.09
Knife Handle	0.77	0.87

Table 18. Adjustment calculations for female DNA recovered from each surface after the secondary domesticated pathway.

Adjustment Calculations – Female DNA				
Raw Data	Adjustment Calculation	Data Adjusted for Loss (ng)	Total DNA Loss (%)	Total DNA Recovery (%)
0.71 ng recovered from domesticated hand	$0.71 = x(0.33)$	2.15	67	33
0.19 ng recovered from mock hand	$0.19 = x(0.33)$	0.57	67	33
0.24 ng recovered from knife handle	$0.24 = x(0.31)$	0.77	69	31
	Total	3.49 ng	38.8%	

Table 19. Adjustment calculations for male DNA recovered from each surface in the secondary domesticated pathway.

Adjustment Calculations – Male DNA				
Raw Data	Adjustment Calculation	Data Adjusted for Loss (ng)	Total DNA Loss (%)	Total DNA Recovery (%)
0.82 ng recovered from domesticated hand	$0.82 = x(0.33)$	2.48	67	33
0.36 ng recovered from mock hand	$0.36 = x(0.33)$	1.09	67	33
0.27 ng recovered from knife handle	$0.27 = x(0.31)$	0.87	69	31
	Total	4.44 ng	49.3%	

A direct transfer during the handshake showed similar quantities of DNA transfer. During the handshake, 33.9% of female DNA transferred onto the mock hand and 53.5% of male DNA transferred onto the domesticated hand (Table 16). Then, after the handshake, the mock hand transferred a total of 0.51 ng of DNA onto the knife handle, 47.5% of it being female DNA (Table 20). This data supports an indirect transfer of female DNA onto the knife handle, as well as a direct transfer of DNA between two identical mock hand substrates. The degradation index ranged from 0.13 – 0.92 with an average of 0.39 showing no degradation throughout this pathway.

The transfer of male and female DNA onto the knife handle when using domesticated fingerprints resulted in lower overall recovery rates than the wild counterpart. Male DNA had higher recovery rates in 11/20 replicates, creating a difficult comparison to identify direct or

indirect transfer (Figure 11). Cell clumping can also affect how the cell suspension is dispensed, because it creates areas of the solution higher in cell quantity and other areas are very low in cell quantity. This can be caused by improper vortexing of the domesticated fingerprint before dispensing onto the mock and domesticated hand. In results of the secondary domesticated fingerprint, an improper cell suspension may have led to lower recovery amounts overall, and more variation between samples producing inconsistent results.

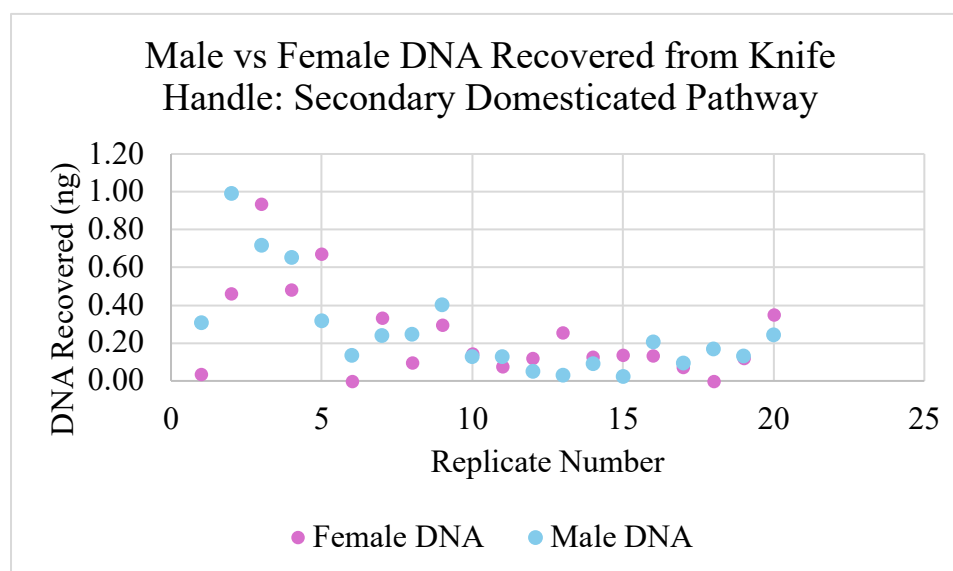


Figure 11. Female and male DNA recovered from the knife handle after a secondary domesticated pathway took place.

The standardized values of each surface highlight the variance in samples (Table 20). The domesticated hand and the knife handle both have standard deviations slightly lower than the mean, while the mock hand has an equal standard deviation to the mean. All three surfaces had samples that varied more than expected, but the mock hand had the most sample variation of the three. When compared to the secondary wild standardized values, the domesticated pathway does show a lower ratio, but the difference is not large enough to eliminate variation between samples.

Table 20. Standardized values of total DNA recovered from the three surfaces in the domesticated secondary pathway.

Total DNA (ng)	Domesticated Hand	Mock Hand	Knife Handle
Mean	1.54	0.55	0.51
Range	0.25 – 4.84	0.08 – 1.96	0.12 – 1.66
SD	1.48	0.55	0.46

Five secondary domesticated samples from the knife handle were amplified and sequenced, and four produced profiles (Figure 12). There was a small range of how many loci were able to be analyzed, spanning from 1 – 11. Low sample concentration may have impacted the profile results, lowering the number of loci. In the secondary pathway, shared alleles are noticed throughout each profile. The sample from knife 2 shows a mixed profile of 22 alleles with 7 attributed to the male and 8 to the female, but 7 alleles are also shared between the two. This makes it difficult to discern the major and minor contributor, as the shared alleles cannot be assigned to either the male or female. Knives 1, 3, and 4 produced 7, 5, and 6 alleles respectively with no female reference identified, only shared alleles between the two references. In these profiles it is not possible to determine a minor or major contributor or identify mixtures. Sample quantity also correlated to allele recovery, as knife 2 quantified more DNA than the other four. In the secondary domesticated pathway, high quantitation of sample recovery increased allele number in profiles. Out of four profiles, three of them had a majority of male alleles present, but also contained multiple shared alleles, and does not identify a major or minor contributor.

A representative profile recovered from knife 2 is shown in Figure 13. It identifies which allele peaks are attributed from either the male or female reference. The asterisk represents peaks that were not identified as allele sites or fall under the threshold.

LOCI	FEMALE 1 ALLELE	MALE 1 ALLELE	KNIFE 1	KNIFE 2
Amel	X	X Y	X	X Y
D3	P Q	YY O	N/A	O P Q
D1	P Q	RR	L RR	P RR
D2S441	PP	N O	N/A	PP
D10	M O	L P	P	N/A
D13	H K	J K	N/A	N/A
PentaE	K L	G L	N/A	N/A
D16	K L	K L	N/A	K L
D18	O R	M N	N/A	M N O
D2	T Y	W Z	N/A	N/A
CSF	K L	J K	N/A	N/A
PentaD	I L	K L	N/A	N/A
THO1	G H	F G	N/A	F G H
vWA	P Q	Q R	N/A	P Q
D21	CC	BB	N/A	N/A

	DD	XX		
D7	H	J	N/A	N/A
		L		
D5	L	I	N/A	N/A
		K		
TPOX	H	H	N/A	N/A
DYS391	N/A	J	N/A	N/A
D8	M	M	N	M
	N	N		N
D12	O	P	P	ZZ
	R	ZZ		R
D19	M	L	N/A	N/A
	N	M		
FGA	V	W	N/A	N/A
	W	Z		
D22	K	K	K	N/A
	P			

	FEMALE 1	MALE 2		FEMALE 1	MALE 3		
LOCI	ALLELE	ALLELE	KNIFE 3	ALLELE	ALLELE	KNIFE 4	KNIFE 5
Amel	X	X Y	X	X	X Y	X Y	N/A
D3	P	N	N	P	N	N	N/A
	Q	O	O	Q	P	P	
D1	P	P	N/A	P	AD	N/A	N/A
	Q	ZZ		Q	ZZ		
D2S441	PP	J	N/A	PP	K	N/A	N/A
		K			N		
D10	M	M	N/A	M	O	N/A	N/A
	O			O			
D13	H	H	N/A	H	J	N/A	N/A
	K	L		K	M		
PentaE	K	K	N/A	K	L	N/A	N/A

D16	L	L	N/A	L	M	N/A	N/A
	K	J		K	L		
	L	K		L	M		
D18	O	L	N/A	O	L	N/A	N/A
	R	Q		R	N		
D2	T	S	N/A	T	S	N/A	N/A
	Y	W		Y	V		
CSF	K	J	N/A	K	L	N/A	N/A
	L	K		L			
PentaD	I	I	N/A	I	I	N/A	N/A
	L	L		L			
THO1	G	F	N/A	G	F	N/A	N/A
	H	G		H	H		
vWA	P	Q	N/A	P	P	N/A	N/A
	Q	R		Q	S		
D21	CC	CC	N/A	CC	CC	N/A	N/A
	DD	DD		DD	WW		
D7	H	H	N/A	H	J	N/A	N/A
		G					
D5	L	K	N/A	L	K	N/A	N/A
		L			M		
TPOX	H	H	N/A	H	H	N/A	N/A
					K		
DYS391	N/A	K	N/A	N/A	J	N/A	N/A
D8	M	J	J	M	J	M	I
	N	O		N	M		
D12	O	ZZ	X	O	R	N/A	N/A
	R	V		R	U		
D19	M	M	N/A	M	M	M	N/A
	N	O		N	N		
FGA	V	X	N/A	V	T	N/A	N/A
	W			W	X		
D22	K	P	N/A	K	O	N/A	N/A
	P			P	P		

Figure 12. Allele table of five knife handle samples after a secondary domesticated pathway. Gray alleles are unmatched to the references, and the red alleles are shared by both references.

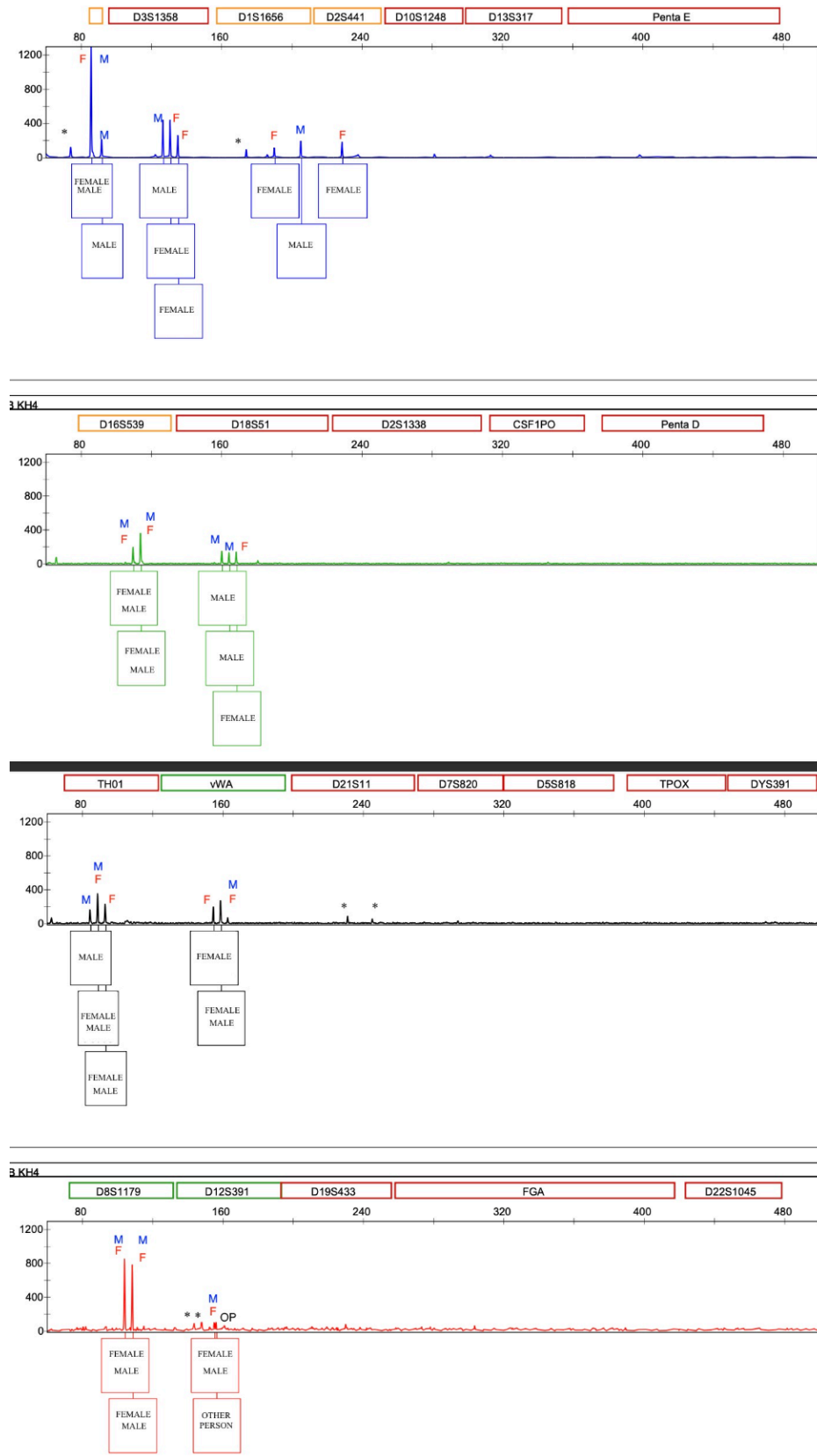


Figure 13. Profile produced from a knife handle sample after a secondary domesticated pathway.

3.3 Statistical Analysis

All four pathways were compared using a one-way ANOVA test to determine if the type of transfer statistically affected the amount of DNA recovered. The p-value corresponding to the F statistic, was lower than 0.05 showing that the four pathways are statistically significantly different (Table 21). Due to the quantified differences from the knife handles in each pathway, this confirms the pathways as being noticeably different from one another.

Table 21. One way ANOVA results of each pathway compared against each other.

Source	Sum of Squares (SS)	Degrees of Freedom (ν)	Mean Square (MS)	F Statistic	p-value
Treatment	53.0695	3	17.6898	15.3075	7.30E-08
Error	86.6724	75	1.1556		
Total	139.7419	78			

The ANOVA test showed a difference between all the pathways, and a Tukey HSD post-hoc test was used to compare individual pathways. This test used the previously analyzed results of the p-value (7.30E-08) and the total degrees of freedom for error (75) to calculate critical values. The difference between groups is recognized as either statistically significant or insignificant (Table 22).

Table 22. Tukey HSD test results comparing all four pathways in individual comparisons. Results show a statistical significance between primary wild and domesticated, primary domesticated vs secondary wild, and primary and secondary domesticated.

TREATMENTS PAIR	TUKEY HSD Q STATISTIC	TUKEY HSD P-VALUE	TUKEY HSD INFERENCE
PRIMARY WILD VS DOMESTICATED WILD	7.197	0.0010053	** p<0.01
PRIMARY WILD VS SECONDARY WILD	0.1412	0.8999947	insignificant
PRIMARY WILD VS SECONDARY DOMESTICATED	1.2315	0.796923	insignificant
PRIMARY DOMESTICATED VS SECONDARY WILD	7.4341	0.0010053	** p<0.01
PRIMARY DOMESTICATED VS SECONDARY DOMESTICATED	8.5386	0.0010053	** p<0.01
SECONDARY WILD VS SECONDARY DOMESTICATED	1.1045	0.8468119	insignificant

Results of the Tukey HSD test showed significance in 3 pairs of pathways: primary vs domesticated wild, primary domesticated vs secondary wild, and primary vs secondary domesticated. These three pathways are likely to be different from each other, and the results are not due to chance. However, the remaining three pairs of pathways are insignificant meaning the results of the means from these pathways are likely due to chance and not the variables affecting the pathway. The significant results from the primary and secondary domesticated pathways confirm a difference in average recovery and the ability to identify a direct or indirect transfer. However, the primary vs secondary wild results are insignificant and the null hypothesis of

transfer not affecting recovery of DNA is not able to be rejected. This is also true for the primary wild vs secondary domesticated and the secondary wild vs domesticated pathways.

The comparison of secondary wild and domesticated pathways is confirmed with a *t*-test. The difference in indirect transfer of touch DNA between wild (mean: 0.77 ng, SD: 0.87) and domesticated (mean: 0.51 ng, SD: 0.46) fingerprints was not statistically significant ($t(29) = 1.209$; $p < 0.236$).

Finally, primary pathways were also confirmed using a *t*-test to identify any significance between wild and domesticated fingerprints. The difference in direct transfer of touch DNA between wild (mean: 0.81 ng, SD: 1.30) and domesticated (mean: 2.56 ng, SD: 1.41) fingerprints was significant ($t(37) = -4.03$; $p < 2.66E-04$).

4. Conclusion

4.1 Wild Pathway Comparison

The pathways conducted in this study focus on direct and indirect transfer mechanisms. The difference between primary contact and secondary contact are noticeable in the amount of DNA recovered on the end surface. Primary pathways, representing direct contact, were reliable in identifying a female as the last subject to hold the knife, even with some samples containing small amounts of male DNA. Direct transfer of DNA would be expected from the larger quantified source on the end surface. A profile showing a major contributor is expected to be the source of DNA directly transferred onto the knife handle. Variation in shedder status and common spaces can influence the amount of other DNA being detectable in a sample. The shedder status played a large part in the evaluation of the secondary pathway in this study.

The indirect contact of female DNA on the knife handle was undetectable in half of the replicates. In most of the other half, samples contained very low amounts of female DNA and higher amounts of male DNA, identifying a male as the last subject to touch the knife. This was solidified by the male being a major contributor in the profile sample. Studies have shown the likelihood of DNA recovery from the palm to be much lower than the fingertip, even when from the same individual (Oleiwi et. al 2015). This could be a significant source of impact on the amount of DNA recovered directly from female hands, as well as the transfer onto male hands. The sampling area of the knife handle was small, due to the direct contact with the secondary fingertip, and could have impacted the amount of DNA recovered as well. Secondary transfer of DNA is expected to be recovered in low amounts when compared to the other source of DNA on the end surface (Figure 14).

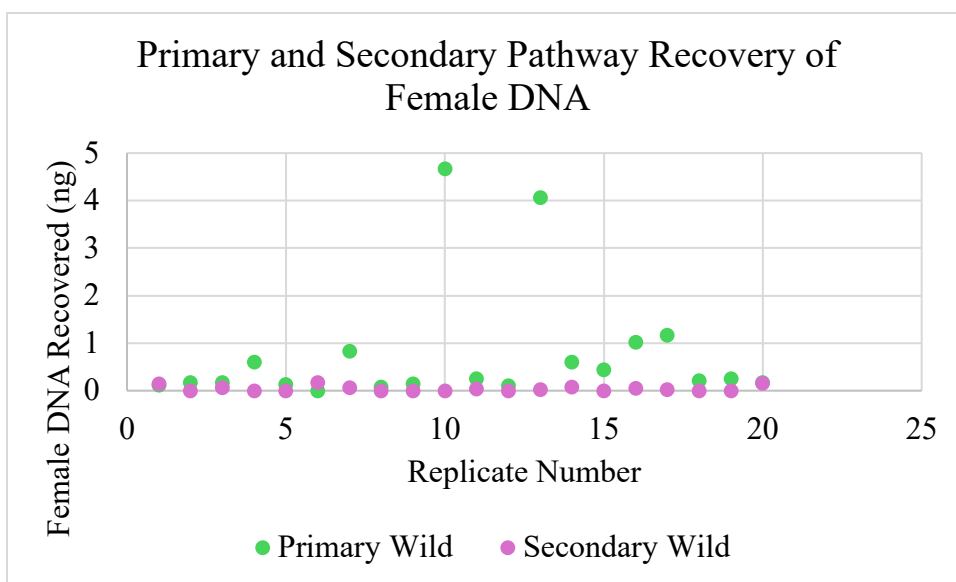


Figure 14. Comparison of female DNA recovered from the knife handle after direct transfer (green) and indirect transfer (pink).

4.2 Domesticated Pathway Comparison

Intentions of creating a standardized positive control in the form of a domesticated fingerprint eliminated the variability of shedder status, handwashing, and other factors influencing the deposition and recovery of touch DNA. However, results of high standard deviation, and unreliable recovery percentages showed error in the prepared domesticated fingerprints. The primary pathway using a domesticated fingerprint resulted in a higher average recovery than the primary wild counterpart. The secondary pathway demonstrated a transfer of DNA with higher amounts of male DNA throughout the pathway but still quantified significant female DNA on surfaces too. In comparison, shedder status affected the secondary wild pathway average female DNA, and it was excluded from the knife handle and otherwise recovered in low amounts. The secondary domesticated pathway emphasizes ability of indirect transfer and potential effect of hand placement on recovery.

Complications from the domesticated fingerprint arise in creation of a cell suspension. The exact amount of DNA likely exceeded nine nanograms in an unknown number of replicates in the primary domesticated pathway, due to cell clumping and improper distribution. This is apparent in DNA recovery percentage from the primary domesticated pathway being higher than 100% (Table 11). Cell clumping also contributed to a lower estimate of cells in domesticated fingerprints used in the secondary domesticated pathway, as DNA recovery percentages were lower than 50% (Tables 18 and 19). However, a domesticated fingerprint is a reproducible positive control. It can be used in different pathway formats with different activities and end surfaces for data collection.

Samples collected in the secondary pathway were from a non-personal item, the knife handle, that had been continuously decontaminated in between replicates. DNA recovered in this pathway showed an almost even quantity from male and female sources, even though the male was last to handle the knife. Van Oorschot et al. (2019) identifies the difference of personal versus non-personal items and the persistence DNA has in each scenario. A non-personal item, such as the knife handle, would be expected to recover a mixture profile after being touched by multiple people. However, they note dependence of shedder status and frequency could impact the mixture profile and the last person to handle the object might not be the major profile (van Oorschot et al. 2019). Participants had a low frequency of handling the knife and used it for 10 seconds. An expected profile from the secondary domesticated pathway might contain more shared alleles between references instead of showing a mixed profile. The quantity of DNA recovered is also variable and difficult to differentiate when combined with the low ratio of standard deviation to mean. Although small, there is still a difference in quantitation, and male DNA had a higher recovery and corresponded to the last person directly holding the knife.

4.3 Overall

The impact of variability on touch DNA recovery creates a need for understanding potential affecting factors. Objects and individuals, positively or negatively, impact touch DNA deposition and recovery under different circumstances. Variable quantity increases when combined in a scenario where DNA is transferred directly or indirectly. A bullpen of standardized values is imperative to quantify the true amount of touch DNA recovered. Surfaces with impacting retention values included a Puritan® sterile swab, organic extraction with phenol:chloroform:isoamyl alcohol, Lorica leather in the form of a mock hand, and a knife handle. Retention of DNA varied between 10 – 67% depending on the pathway, greatly affecting the amount of touch DNA able to be recovered.

Each pathway involving participant activities, such as handwashing and a handshake, is a reliable method on establishing an activity-level proposition. Results of direct transfer show a major contributor to the profile recovered from the final surface, and a higher quantitated value when comparing female and male DNA. Indirect transfer also shows the last person to touch the end surface, even after engaging in a handshake with someone else, as the higher quantitated DNA value. Although, the ability of DNA to be indirectly transferred onto the end surface is plausible, it is highly variable in wild fingerprints sometimes resulting in undetectable amounts.

Tracking touch DNA throughout an unknown pathway can be compared to data gained from known activities, developing a reliable conclusion of DNA transfer in real-life scenarios. Sample collection and deposition of touch DNA on hands may impact the amount deposited. This study replicated real scenarios involving wild fingerprints and reproduced them in a laboratory setting using a known positive control to track the touch DNA across direct and indirect pathways. Efforts to create a domesticated fingerprint with a reliable starting quantity of

nine nanograms resulted in variable data that concluded to be from improper cell clumping. Variables of sample processing were standardized and solidified the notion that direct and indirect transfer of touch DNA is possible and showed the last person to touch an object as the significant source of DNA.

4.4 Future Research

Different factors affecting touch DNA transfer can be exchanged to create new pathways simulating real-life scenarios. Research regarding the recovery rates of different methods of collection and sample processing are useful to the expanding knowledge of how touch DNA samples should be handled. New pathways can be created using a domesticated fingerprint, highlighting the different methods of transfer, such as quaternary transfer between three or more individuals. A person's DNA can be transferred to an object without direct contact, and with addition of data studying effects of transfer, it can be easier to understand touch DNA evidence.

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