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Evaluating a Test for Shedding Propensity Using Tape Lifts from Different Skin Locations

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Abstract

The shedding propensity of a person can assist data interpretation in casework when assessing the possibility of passive transfer for DNA analysis. Past studies on shedding propensity evaluated palmar skin (washed and unwashed) deposits. This study compared different skin locations with respect to shedding propensity, and explored the potential of tape-lifts as a skin surface collection method. Eight different skin types and samples were collected with adhesive tape disks from 28 participants over three non-consecutive days; the washed and unwashed fingers from both hands, toe, and arm, neck below ear, and nape. Samples were extracted, quantified, amplified, genotyped, and evaluated for the presence of DNA mixtures. Concentration for DNA mixtures were then modified by subtracting the non-donor DNA percentage. DNA concentrations for different skin sample types were log-transformed and tested for Pearson r correlation values. Of all the sampling locations tested, left and right washed fingers were the most suitable to predict shedding propensity. Despite lower DNA concentrations than for sebaceous and unwashed finger samples, washed fingers were the most consistent over the three collections, and provided good shedding propensity division within donors. A preliminary model was made to predict shedding propensity. Only for left washed fingers of “high shedders” the prediction for an individual remained unchanged over all three weeks, while for other locations and “medium” and “low” shedders predictions were inconsistent. Tape lifts from washed fingers seem to be a possible tool to determine shedding propensity, and further work will be needed on sampling other areas.

Introduction and Literature Review

Forensic relevance of trace DNA

Human DNA recovered from items and surfaces at the crime scene plays a vital role in court, if it links the person of interest to the crime committed. Touch DNA, also known as trace DNA, may be left at a crime scene (Van Oorschot et al., 2010). The advancement of techniques currently enables laboratories to obtain interpretable data from trace DNA, even when the concentration is below previously established thresholds. Forensic laboratories will test DNA evidence by amplifying polymorphic Short Tandem Repeat (STR) sequences, and measuring their lengths; an STR profile is an electropherogram showing STR allele sizes at different loci (Butler, 2015). Evidence STR profiles can be entered in the FBI Combined DNA Indexing System (CODIS) database and compared to the STR profiles of the suspects, victims or other persons of interest. When the DNA sample has more than one donor, the profile would appear to have multiple peaks on the same locus, indicating a mixture profile. For contact trace DNA such mixed profiles may complicate an accurate analysis to determine which donor was the depositor (Van Oorschot et al., 2010).

When a person has direct contact with the surface or an object, there is direct or active transfer of DNA onto the object. However, if the transfer involves DNA from another individual, i.e. foreign DNA, on the hand of the depositor or an object they are holding, the foreign DNA would be indirectly deposited onto the object, and called passive transfer (Fonneløp et al., 2017). In such situations, the STR profile of both the handler and the contributor of the foreign DNA could appear as a mixture, and there could be disputes on who is the active DNA depositor to the object, e.g. a weapon, in

court. The depositor, the one who actively touched the object, is usually the major contributor to a mixture (Farmen et al., 2008). In some cases, the DNA of the active handler might not be observed in an STR profile (Fonneløp et al., 2017), this happens when the depositor did not leave a detectable amount of DNA in comparison to the donor of the foreign DNA. One of the factors that dictate the amount of detectable DNA after contact is the shedding propensity of the donor, and the profile of two contributors is influence by the different "shedding ability" of both (Szkuta et al., 2017).

Sources of DNA in contact traces

Human skin is the largest organ, is protected by an oily layer of corneocytes, and has two types, palmar and sebaceous (Van Smeden et al., 2014). Palmar, or thick and hairless skin, is the friction ridge containing skin on the palms of the hands and soles of the feet; it has eccrine sweat glands. Sebaceous skin is also known as thin and hairy skin and covers the rest of the body. It contains eccrine sweat glands located throughout, as well as apocrine sweat glands limited to the armpit or groin areas, and sebaceous glands mostly connected to hair follicles. Sebaceous secretions are called sebum and consist of glycerides and fatty acids and other substances like wax esters and squalene (Giacomoni et al., 2009). All skin secretions have been shown to contain cell free DNA, which thus plays a role in obtaining DNA results from contact traces (Vandewoestyne et al., 2013). Other sources of DNA from skin include anucleate and nucleated cells, either from the person (self-DNA) or another individual (foreign DNA) (Burrill et al., 2019). It is important to study secretions like sebum and sweat, Ostojic et. al (2014) concluded that the amount of skin debris on fingerprints does not correlate with the ability to produce a good STR profile. This indicates that besides visible cells, cell free DNA is a source of

detectable DNA. Lacerenza et al. (2016) tested swabs and stubs taken from the palmar surface of the hands and fingers from 60 volunteers for the presence of mRNA indicative of body fluids like blood, semen, vaginal mucosa, and the skin specific markers corneodesmosin and loricrin. They found non-skin markers expressed on 15% of their samples. The amount of DNA recovered for these samples was higher than for the skin-only samples. This confirms that the DNA detected on unwashed hands could be from different sources of the body. Personal behavior such as face-touching, or transferring DNA from saliva or sebum to hands, can contribute to higher DNA detection on unwashed palmar skin (Lacerenza et al., 2016, Zoppis et al., 2014). Therefore, unwashed skin samples are not reliable to determine shedding propensity.

Shedding propensity

In the earlier studies, Lowe et al. (2002) defined donors whose touch DNA samples could produce a full STR profile at a controlled time after handwashing as a "good shedder" compared to the ones who could not "poor shedders". With poor shedders only leaving partial DNA profiles behind, the probability of poor shedders depositing detectable DNA is lower than the probability of good shedders. Fonneløp et al. (2017) found that after active contact in simulated attacks the DNA from high shedders was detected in 20 out of 20 samples, with low shedders only being detectable in 15 out of 26 samples. This could also be an issue when analyzing a mixture profile, where the good shedder may show up in the STR profile regardless, if the contributor was the active depositor or not. A study on DNA transfer to objects after a controlled hand shake, had 12 pairs shake hands and then either press their right hand on a glass plate immediately (six pairs, 12 hand prints) or after 15 minutes of activity (six pairs 12 hand prints). Seven

out of 12 immediate samples and three out of 12 delayed samples showed DNA from the handshake partner (Szkuta et al., 2017). Knowing the shedding propensity of a person of interest, would provide useful information for the interpretation of the case when analyzing mixture profiles on the evidence. A passive transfer scenario brought up by the defense may be more likely, if the person of interest can be shown to be a good shedder and often leave detectable DNA behind.

Recent papers divided shedding propensity into three different categories, low, medium, and high for their volunteers. Each have their own methods of determining shedding propensity, including direct swabbing on hands and surfaces of tubes or glass slides after contact as a method to collect DNA (Burrill et al., 2019). Fonneløp et al. (2017), after swabbing conical tubes held by volunteers, defined donors that could produce a good profile and DNA concentration higher than their average as high shedders, otherwise donors were low shedders. Allen et al. (2008) splited the shedding group into three, light, intermediate, and heavy through the relative DNA concentration recovered, and profile quality among the volunteers after swabbing touched glass slides. Oleiwi et al. (2015) compared finger and palm DNA recovered by directly swabbing the hands, and found that DNA recovery from fingers was higher than the recovery from the palms. With a similar method, the results obtained by Goray and Oorschot (2021) show that shedder status was not distinguishable with a direct swabbing of the palm when compared to handprints on glass plates. De Bruin et al. (2012) compared double swabbing and tape-lifting methods, where they swabbed twice on the surface and used double-sided-tape, showing that DNA collected by both of these methods showed alleles. These studies highlight the lack of a standardized definition of shedding propensity.

Kanokwongnuwut et al. (2018) explored a different method by visualizing deposited cellular materials on slides with a fluorescent microscope after staining with Diamond Dye (Promega). Here the volunteers pressed their hands on the glass slides over four different time intervals after handwashing and the prints on the slide were then visualized under the microscope for cell-counts per mm^2.They saw differences among volunteers, and found that the cell-count is correlated with the peak height on their associated STR profiles. However, a similar study concluded that this method was not as "reproducible" as stated (Small-Davidson et al., 2020). The difference between these two studies is that one directly performed PCR with the DNA swabbed off the glass slide without DNA extraction, while Small-Davidson et al. (2020) compared the concentration of the DNA extracted from a finger tape-lift to the cells-count from a glass print that was donated by the same donor on the same day.

The methods described above (Small-Davidson, et al.; 2020) used both unwashed and washed hands to determine shedder status. Prediction of shedding propensity is more difficult when collecting unwashed palmar samples, since there could be different foreign sources of DNA detected on unwashed hands. Therefore, it is important to have the donors wash their hands prior to sampling, which would ensure the sample do not contain non-self-DNA. While most donors are capable of voluntarily washing their hands and being cooperative for the sample collection, it would be difficult to collect samples for uncooperative suspects. The result of the unwashed hand sample collection might include foreign DNA and which could impact the prediction for shedding propensity and analysis. In these scenarios, it would be helpful to find a location that allows analysts to

collect a sample and best represent the shedding propensity of the person without the need for handwashing.

To obtain the data from most of the studies described above, volunteers were required to donate their samples multiple times, over different days, or period of times within a day, and before or after handwashing to predict their shedding propensities (Burrill et al., 2019). This would be inconvenient for the person of interest or donor to pay multiple visits to donate their samples, especially for uncooperative donors and deceased victims. Therefore, it would be beneficial to find a sampling location that allow for a single collection and the best representation the shedding propensity of the donor.

Research Design and Study Goal

A preferred sampling location should provide a consistent amount without requiring multiple collections, and definitively categorize each donor as low, medium, or high shedder. Sebaceous skin should be explored as a possible collection site. This skin type may provide a comparable amount of DNA as the palmar skin to determine shedder propensity. It may provide detectable DNA for an STR profile, and would be less likely to have DNA from other sources, or foreign DNA.

Instead of swabbing surfaces, which introduces multiple variables, or directly on hands, which also can vary in pressure and area covered, tape-lifts could be used to collect DNA samples on skin. A type of adhesive tape is the D-Squame disks, which are routinely used by dermatologists to collect skin samples for clinical tests (Prinz et al., 2019). These discs have uniform circumferences and sizes that would allow for a consistent area of DNA sample collection. They can be applied to uncooperative donors.

However, shedding could be a transient property, changing from day-to-day or dependent on personal habits (Taylor et al., 2016). Collecting samples over several collections would determine the reproducibility of the collection and the sampling location.

This study tested the possibility of using tape discs to predict shedding propensity and explored the possibility of selecting a sebaceous location for self-DNA collection. The goal was to determine the best sampling area to determine shedding propensity. We also tested for reproducibility by repeating each collection three times for three consecutive weeks. The three sebaceous skin sampling locations selected for the study were upper inner arm, nape (the area behind the neck), and ear (the neck area behind the earlobe). The palmar skin sampling locations were toe, left-unwashed index and middle fingers (LU), right-unwashed index and middle fingers (RU), left-washed thumb (LW), and right-washed thumb (RW).

The variance for the DNA concentrations of these sampling locations amongst the group of donors and over the three collections would determine which location best demonstrates different levels of shedding propensity. The lowest variance between collection days indicates the highest reproducibility, and would allow for one-time sample collection. The locations with a high variance among the donors would be preferred since they could provide a distinctive category for the donors without placing all into one group. Lastly, shedding propensity of the donors will be predicted with a preliminary prediction model based on Bayesian Statistics.

Materials and Methods

Sample Collection:

The sample collection process was approved by the CUNY Institutional Review Board (IRB #2018-0099). Thirty volunteers composed of fifteen females and fifteen males were recruited using flyers, two of the thirty donors were excluded from data analysis due to problems with generating STR profiles for these samples. Each volunteer donated their samples after signing an informed consent form. All samples were anonymized and coded immediately after collection. Samples were collected on three separate days, within three weeks. On the first collection day, saliva samples were collected from the volunteer via a buccal swab. On each collection day, the volunteers donated eight skin surface samples using D-Squame tape disks (Cuderm, Dallas, TX). The skin surface samples were collected by lightly placing the tape disks on six skin areas of the volunteer: left big toe, upper inside area of the arm, nape, neck area behind the ear, left hand (index and middle fingers), and right hand (index and middle fingers). Afterwards, the volunteers were asked to clean their hands using only water, dry them with a clean paper towel, and wait thirty minutes. During the thirty minutes, the volunteers were inactive and did not touch anything. After the wait, skin surface samples were collected from the right and left thumbs. For all samples, the tape disks were placed and removed using clean tweezers. All tape disk samples were placed in 2 mL Eppendorf centrifuge tubes (Hamburg, Germany) and stored in the freezer at -20^{o}C. The buccal swabs were stored in brown envelopes at room temperature.

Extraction:

The tape disk samples were extracted using the QiAmp Investigator extraction kit (Qiagen, Germantown, MD). An extraction negative control was prepared for each sample batch. The extraction involved three different steps: lysis, substrate removal, and purification. For the lysis step, the samples were incubated at 56^{o}C for two hours in a buffer solution containing 600 µL of animal tissue lysis (ATL) buffer, 20 µL proteinase K (both from Qiagen, Germantown, MD) and 24 µL dithiothreitol (DTT) (Promega, Madison, WI). Each tape substrate was removed from the centrifuge tube with a cleaned tweezer. In order to remove the adhesive to prevent robot tip clogging during the purification step, the tubes were placed in the -20^{o}C freezer for 4 minutes and centrifuged at 20,000 RCF for five minutes. Two layers were formed. The top layer contained the desired solution, and the bottom layer contained the adhesive. The supernatant was placed into a new 2 mL tube and positioned into the QiaCube (Qiagen, Germantown, MD) for purification, while the bottom layer was discarded. The purification step was performed on a QiaCube automated extraction robot, which has a capacity of twelve samples per round.

The protocol performed by the QiaCube was adapted from the Qiagen Investigator Surface and Buccal Swab Purification script and modified to optimize DNA recovery. Table 1 shows the original procedure on the Qiacube and the customized changes. After the purification, the DNA extracts were stored in the freezer at -20^{o}C.

Table 1. QiaCube protocol

Steps	**Procedure programs**	**Revisions**
Incubation	600 µL AL buffer added	
	Incubated for 300 seconds with 1000 rpm shake	
Bind	300 µL ethanol added and mixed	Original procedure called for 250 µL
Transfer and centrifuged twice	700 µL lysate transferred to the columns	Repeated to accommodate larger lysis volume
	Centrifuged for 90 seconds at 500 rpm	
Washes Wash buffer 1 (AW1) Wash buffer 2 (AW2)	500 µL AW1 buffer added	
	Incubated for 120 seconds	
	Centrifuged for 120 seconds at 7500 rpm	
	680 µL AW2 buffer added	
	Incubated for 120 seconds	
	Centrifuged for 120 seconds at 7500 rpm	
	680 µL of ethanol added	
	Incubated for 120 seconds	
	Centrifuged for 120 seconds at 7500 rpm	
Reposition of Columns	Columns were transferred onto the collection tubes	
Elution: This procedure was performed twice	20 µL of ATE buffer added	Original procedure called for a single addition of 60 µL ATE and 60 second incubation
	Incubated for 180 seconds	
	Centrifuged for 180 seconds with 10,600 rpm	

Quantitation:

Extracted DNA samples were quantified on the QuantStudio 5 Real Time PCR system using Quantifiler Trio and HP Human DNA quantitation kits (all from Applied Biosystems, Thermo Fisher Scientific, Waltham, MA). The calibration curve was prepared using the DNA standard with concentrations of 50, 5, 0.5, 0.05, and 0.005 ng/µL. For each sample, a master mix composed of 5 µL reaction mix and 4 µL primer solution was prepared and added to a 96-well optical plate. Then, 2 µL of each standard, sample, and extraction negative controls was added to the assigned wells. No template controls (NTCs) consisted of master mix without any additions. The plate was sealed, briefly centrifuged, and placed into the QuantStudio 5 PCR system (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA). Reaction conditions are shown in Table 2. Each tape-lift sample was quantified in triplicate. The average of the set was used for data analysis.

Table 2. Quantifiler Trio Real Time PCR Cycling Parameter for 30 Cycles

Initial Incubation	**Denature**	**Annealing**	**Extension**	**Final Extension**	**Final Hold**
HOLD	CYCLE (30)			HOLD	HOLD
95 ºC	94 ºC	59 ºC	72 ºC	60 ºC	4 ºC
11 min	20 sec	2 min	1 min	45 min	∞

Amplification:

Short tandem repeat (STR) amplification was conducted using GlobalFiler PCR Amplification Kit (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA). The DNA positive control was diluted by adding 5 µL of the DNA control to 10 µL of 0.1X Tris-EDTA buffer. 7.5 µL of Master Mix was added to 2.5 µL of the primer set. Fifteen µL of negative control, positive control, and DNA extracts were added to the Master Mix and primer set solution. The optimal DNA amount was 1 ng. The sealed plate was briefly centrifuged and placed in a Veriti 96-Well Thermal Cycler (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA). Reaction conditions are shown in Table 3. The amplified product was stored at 4^{o}C.

Table 3. Globalfiler Trio thermal cycler cycling parameter for 29 cycles.

Initial Incubation	**Denature**	**Annealing**	**Final Extension**	**Final Hold**
HOLD	CYCLE (30)		HOLD	HOLD
95 ^{o}C	94 ^{o}C	59 ^{o}C	60 ^{o}C	4 ^{o}C
1 min	10 sec	90 sec	10 min	∞

3500 Genetic Analyzer:

STR profiles were obtained through capillary electrophoresis on a 3500 Genetic Analyzer (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA). For each sample, a mixture of 0.36 µL GeneScan™ 600 LIZ Dye Size Standard and 11 µL of Hi-Di formamide was added to a MicroAmp Optical 96-Well Reaction Plate ((all Applied Biosystems, Thermo Fisher Scientific, Waltham, MA). Then 1.2 µL of the amplicons,

positive, and negative controls were added to the wells respectively. The injection condition on a 3500 Genetic Analyzer was 1.2 kV for 15 seconds. Data was then analyzed with GeneMarker HID (SoftGenetics, State College, PA).

Volume normalization and foreign DNA adjustment:

Concentrations for samples with lysate remains were adjusted to compensate for the unpurified lysate that were left behind on the Qiacube in the beginning of the project. The remaining lysate's volume had been measured and was used as a multiplication factor to correct DNA concentrations to correspond to the total volume of lysate as if the full volume had been purified. This correction was applied to 90 samples.

The buccal reference STR profiles were used to distinguish self and non-self DNA and categorize each sample either as a single source sample, or as one of three mixture categories as described by Trapani (2021). Mixture types of the tape STR profiles were categorized into three different percentages, 5%, 34%, and 66% of foreign DNA; These percentages were deducted from the average quantitation values and then these adjusted self-DNA concentrations were used for statistical analysis.

Statistical analysis:

The adjusted concentrations of each skin area were converted into log function and compared against each other skin area for Pearson correlations with Microsoft Excel. Correlations with an R value above 0.4 were considered to be significant. The shedder propensity Bayesian prediction model was created with R-studio to predict the shedder status for each volunteer.

Results

DNA results are quantitation values after DNA extraction, triplicate quantitation and non-self DNA adjustment for 772 skin surface samples processed as described in Material and Methods. The eight different sample types include unwashed and washed fingers from both hands, and a toe sample to represent palmar skin and three sebaceous skin areas: upper arm, below ear, and nape.

Sample Type Variation in DNA Concentration:

A whisker plot generated to show average concentration per sample type for all three collections is shown in Figure 1. The x mark indicates the mean of the sample. The figure shows that the ear samples displayed the highest average DNA concentration with the largest range, which was consistent for all three collections. The next highest average was detected for left unwashed fingers followed by the nape. The collection site with the lowest average concentration is the toe, followed by right washed fingers, left washed fingers, and then the arm. Although the left-unwashed-fingers-location had the second highest average, it also has the second widest interquartile range after the ear. The left unwashed fingers sampling yielded the highest concentration of all measured samples with a value of 1.85ng/µL from the triplicates, and the triplicate average for one of the collections was 0.63ng/µL which is the highest average concentration shown as the highest outlier in Figure 1. This unexpectedly high value could be explained if the volunteer might have come in contact with high DNA sources, such as saliva or other bodily fluids**.** Although the toe samples have several high concentrations, they are shown to have the lowest average of all the sample types and the average is not within the box, showing the inconsistency of the collections. Similar to toe samples, left unwashed

fingers also have an unexpectedly high average concentration, making it the second highest in the whisker plot.

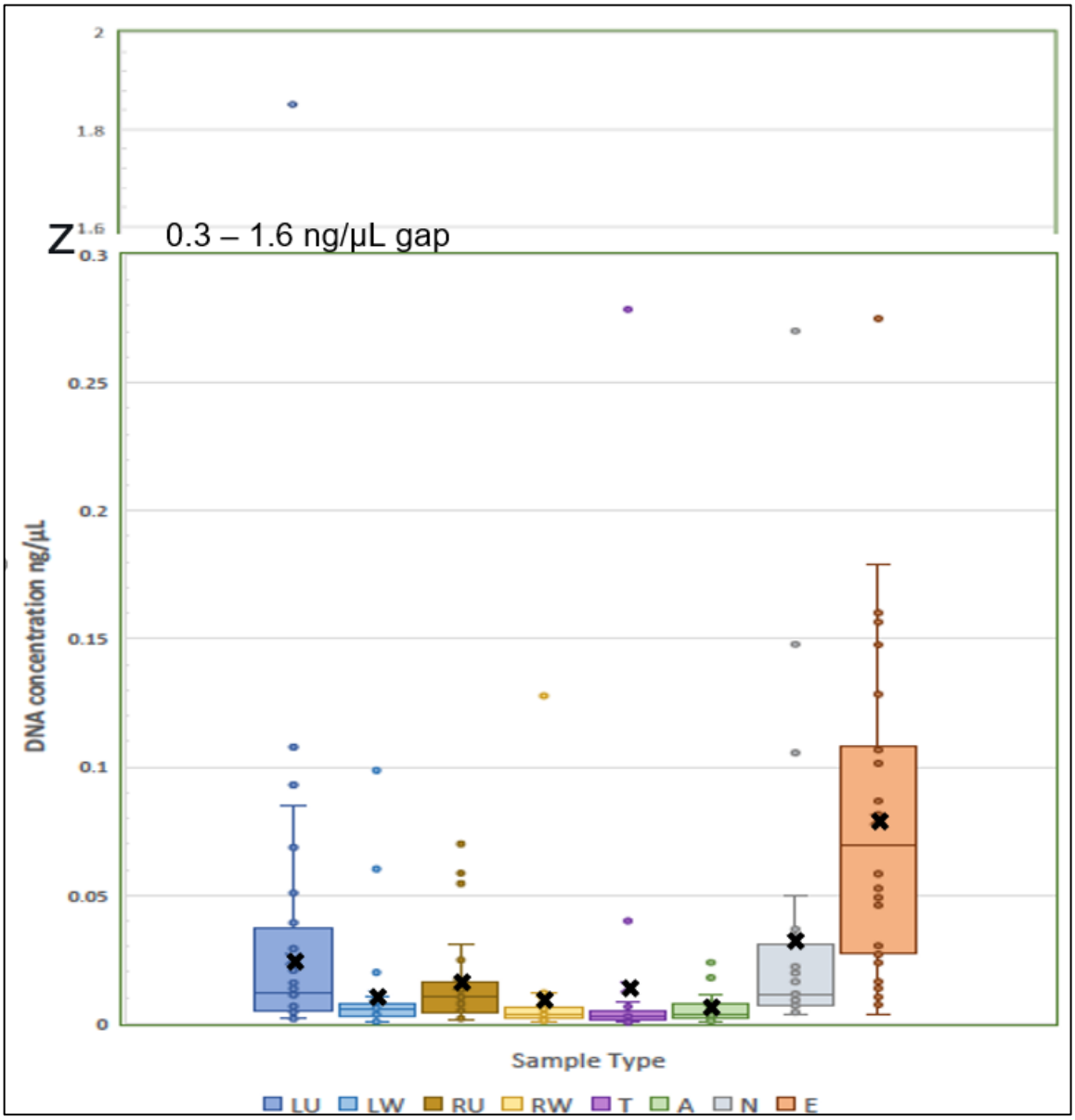

Figure 1: *Whisker plot for 3-day Average DNA concentration per sample type. Horizontal lines depict the median, x the mean, box size the interquartile range, and whiskers the top 25% range excluding outliers. Samples shown are: LU – left unwashed, LW – left washed, RU - right unwashed, RW – right washed, T – toe, E – ear, N – nape, A – arm. n= 84 each.*

Overall, with exception of the unwashed samples palmar skin samples yielded a lower concentration in comparison to sebaceous skin.

Sample type correlations:

The logarithm for the average concentrations of each sample type per collection was used to create Pearson correlation plots for all possible pairing combinations of all sample collection types; an example each for the highest correlated pair and lowest correlated pair is shown in Figure 2. Figure 2a shows the correlation plot for the left unwashed fingers (LU) and left washed fingers (LW) collection location, and the R^2 value is 0.6194 (r = 0.7870), the highest of all the pairs. Figure 2b shows the left unwashed (LU) and ear (E) correlation, having the lowest R^2 value of 0.0003 (r = 0.0173). These R^2 values were generated using R and Excel from the plots for all 28 pairs and were converted to correlation coefficients (r values) which are shown in Table 4.

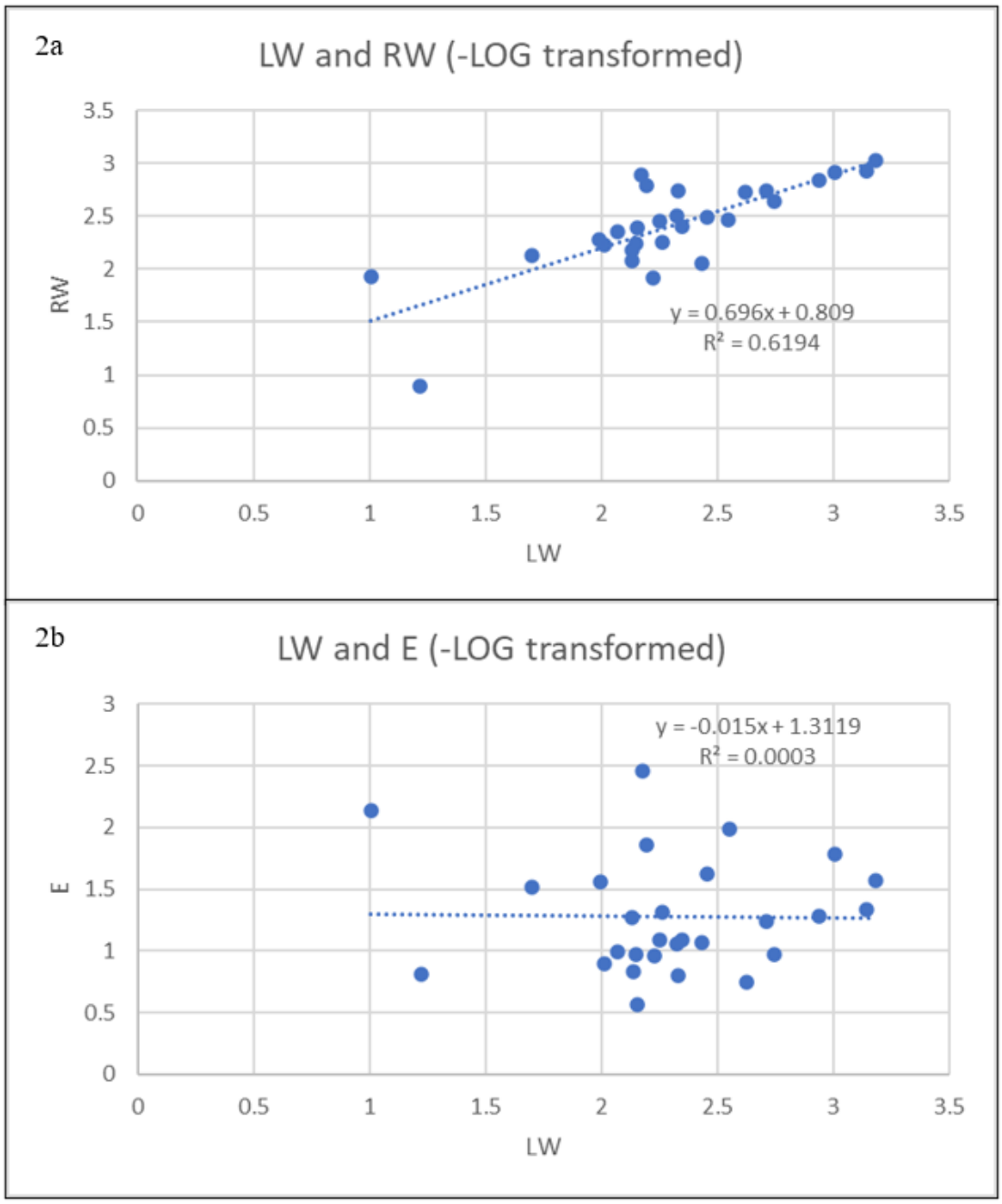

Figure 2 a,b: *Example of Pearson Correlations for log transformed quantitation averages over all three collections; 2a shows the left washed (LW) and right washed (RW) finger calculation with a strong correlation (n=28); 2b shows left washed (LW) finger values plotted against ear (E) values displaying a lack of correlation (n= 28)*

The R values for each combination were obtained for each collection to determine the degree of correlation for each sample pair and for the averaged R values from the three

collection days. Table 4 illustrates arranged overall R values from high to low R values; these R values are from the log of average from all three collections. The peach highlighted texts are all friction ridge or palmar skin collections, teal (arm), blue (nape), and gold (ear) are sebaceous collections. The green bars help to visualize the values and compares each value with other pairs in the data. The highest average R value is 0.79, which is the pair between the washed fingers, and the lowest is between left-washed fingers and ear which is 0.02. If the R value reached >0.4 for a sample pair, then the correlation is considered to be significant. The right half of the table shows if R values reached the 0.4 value for each collection week. The Ns represent NO indicating the R value is lower than 0.4 for that collection, YES or Ys indicate R values equal or higher than 0.4.

The finger collection pairs are consistent over the three collections, and they have R values above 0.4; however, there are two exceptions, one is the left washed (LW) and unwashed (LU) pair and the other being left unwashed (LU) and right washed (RW) collections. Pair LW and LU has an overall R value of 0.75 but has a value under 0.4 in week one. Similarly, week one for pair LU and RW was not consistent with its overall average R value of 0.65.

Table 4: DNA concentration value correlation between sample types*

	Correlation based on Average		value above R = 0.4 (based on log plots)		
1ST	2ND	R	Week 1	Week 2	Week 3
LW	RW	0.79	Y	Y	Y
LU	LW	0.75	N	Y	Y
RU	RW	0.73	Y	Y	Y
LU	RU	0.71	Y	Y	Y
LW	RU	0.66	Y	Y	Y
RU	A	0.65	N	N	N
LU	RW	0.65	N	Y	Y
RW	A	0.60	Y	N	N
RW	N	0.55	N	N	Y
RW	T	0.49	N	Y	N
LU	N	0.49	N	N	Y
T	A	0.47	N	Y	N
RU	N	0.44	N	N	Y
RU	T	0.40	N	N	N
E	N	0.39	N	N	Y
LW	N	0.36	N	N	Y
LU	T	0.34	N	N	N
N	A	0.33	N	N	N
LW	A	0.33	N	N	N
LW	T	0.32	N	N	N
LU	A	0.30	N	N	N
RW	E	0.30	N	N	Y
T	N	0.26	N	N	N
E	A	0.15	N	N	N
RU	E	0.08	N	N	N
LU	E	0.07	N	N	N
T	E	0.05	N	N	N
LW	E	0.02	N	N	N

**First two columns count from the left are the sampling location pairs, key as followed: LU = left unwashed fingers. RU = right unwashed fingers. LW = left washed finger samples. RW = right washed finger samples. A = arm. N = nape. E = ear. T = Toe. The third column are the sorted R values associated with the location pairs. The last three columns indicate the R values above 0.4 over three collections, with N = No for R value not above 0.4, and Y = Yes for R values above 0.4.*

Through the color visualization, it is quite evident that the peach highlighted pairs (palmar skin) are mostly arranged at the top of table 4, while other color combinations are arranged towards the bottom part of the table. Most of the combinations at the bottom consist of sebaceous samples, with the bottommost being the ear location sampling. Additionally, all pairs with ear tape lifts are below an average R value of 0.4, and their individual week R values are also under 0.4 with the exception of the nape and RW on their third collection. The ear and nape pair shows the highest correlation of all sebaceous combinations, while the arm-ear pair is the lowest. Interestingly, the ear samplings show low correlations with all non-sebaceous skin samplings, having R values below 0.4 and it is the only sebaceous sample type that do not yield R value higher than 0.4 with palmar samples combinations. On the other hand, arm pairing with palmar shows a relatively higher correlation compared to other sebaceous combinations with palmar. This confirms data from a pilot project on shedder status determination (Prinz et al., 2019). The highest sebaceous pair with palmar, RU and arm, has an overall R value of 0.65, however, their R values for the three individual weeks are under 0.4.

Aside from correlation between each skin area combinations, proportion of variation for each skin area was obtained with the LME4 package in R and is summarized in Table 5. The donor-donor variation measures how distinct the shedding propensity is between individual donor per sampling location, while daily variation measures the reproducibility of that sampling location and if it would yield the same amount across the collection days.

Table 5: Systematic proportion of variation**

Location	Source of Variation	Variance	SD	Proportion of variation (%)
LU	donor - donor	0.9	0.95	**39.01**
	daily	1.41	1.19	**60.99**
LW	donor - donor	0.82	0.91	**53.27**
	daily	0.72	0.85	**46.73**
RU	donor - donor	0.44	0.66	**34.3**
	daily	0.84	0.91	**65.7**
RW	donor - donor	0.66	0.81	**56.39**
	daily	0.51	0.71	**43.61**
T	donor - donor	0.54	0.74	**24.43**
	daily	1.67	1.29	**75.57**
E	donor - donor	0.66	0.81	**36.3**
	daily	1.16	1.08	**63.7**
N	donor - donor	0.76	0.87	**43.96**
	daily	0.97	0.99	**56.04**
A	donor - donor	0.51	0.71	**42.63**
	daily	0.68	0.83	**57.37**

***Yellow/brown shades indicate the two samples where donor-donor variation is greater than 50% and daily variation is smaller than 50%.*

The RW sampling location shows the highest of the proportion of variation for donor-donor, (56.39 % in table 5), which shows that here shedding propensity differences would be more distinct between different donors compared to other sampling locations. This is followed by LW, with a value of 53.27%. All other sample types, including unwashed fingers, have less than 50% of their variation within the donor-to-donor group. The order is nape with 43.96%, 42.63% for the arm, 39.01% for LU, 36.3% for the ear, 34.3% for the RU, and lastly, the toe with 24.43%. The two locations with the highest proportion of donor-to-donor variation (RW and LW) are highlighted peach in Table 5 while the rest of the locations are in light blue. The sampling location with the highest proportion of

variation in daily variation, is the toe with the percentage of 75.57%, indicating that it is the most inconsistent and least suitable to determine shedding propensity compared to other locations. The second highest is RU with 65.7%, followed by ear with 63.7%, then LU which is 60.99%, arm with 57.37%, nape with 56.04%, lastly, LW with 46.73% and RW with 43.61% which are highlighted in brown in table 5. RW and LW have the lowest proportion variation, therefore, these two locations are more consistent and more suitable for determining shedding propensities in one single collection compared to other sampling locations.

Prediction model for shedding propensity:

For each sampling location, a plot of 28 donors sorted based on the logs of DNA concentrations from low to high was generated in R studio. Figure 3 shows the left washed-fingers location, an example of the sorted plots, where each point represents one donor with their associated log DNA concentration, with data points arranged by increasing log of DNA concentrations of the 28 donors. Then, using the package ggplot in R Studio, a hundred data points were simulated for each sampling location plot as shown in figure 4 for left washed fingers; these simulated points were added to the original sorted donors. Then, a change point analysis was applied to estimate the cutoffs for the three categories.

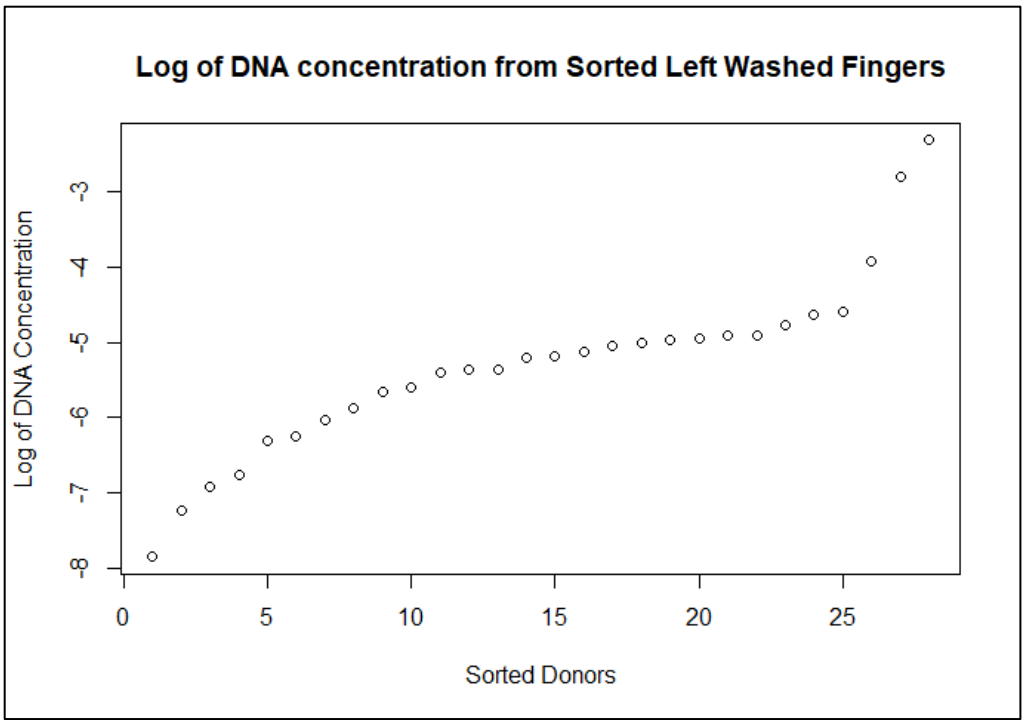

Figure 3 *Example for sorted DNA concentration – left-washed fingers. Donors sorted from low to high log DNA concentration. n=28.*

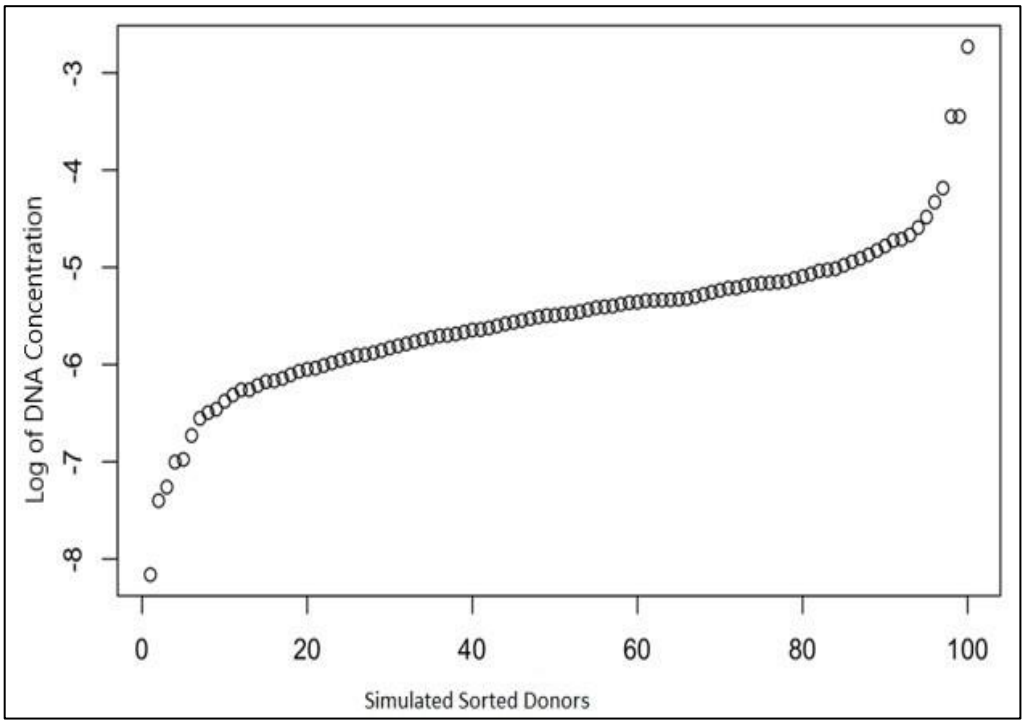

Figure 4: *Simulated plot for 100 donors – left-washed fingers: 100 simulated donors sorted from low to high log DNA concentration. n=100.*

Figure 5 is an example of a change point analysis model and is showing the left-washed location; the left tau is indicated by the darker dot at around the 20th simulated donor where it detected the highest chance of a change in the distribution, which at that point divides low and medium shedders, The right change point, likely dividing medium and high shedders is marked as the darker dot at the 80th donor. This model was used to predict the probability of which shedding propensity category each donor would belong to by inputting their log transformed DNA concentrations.

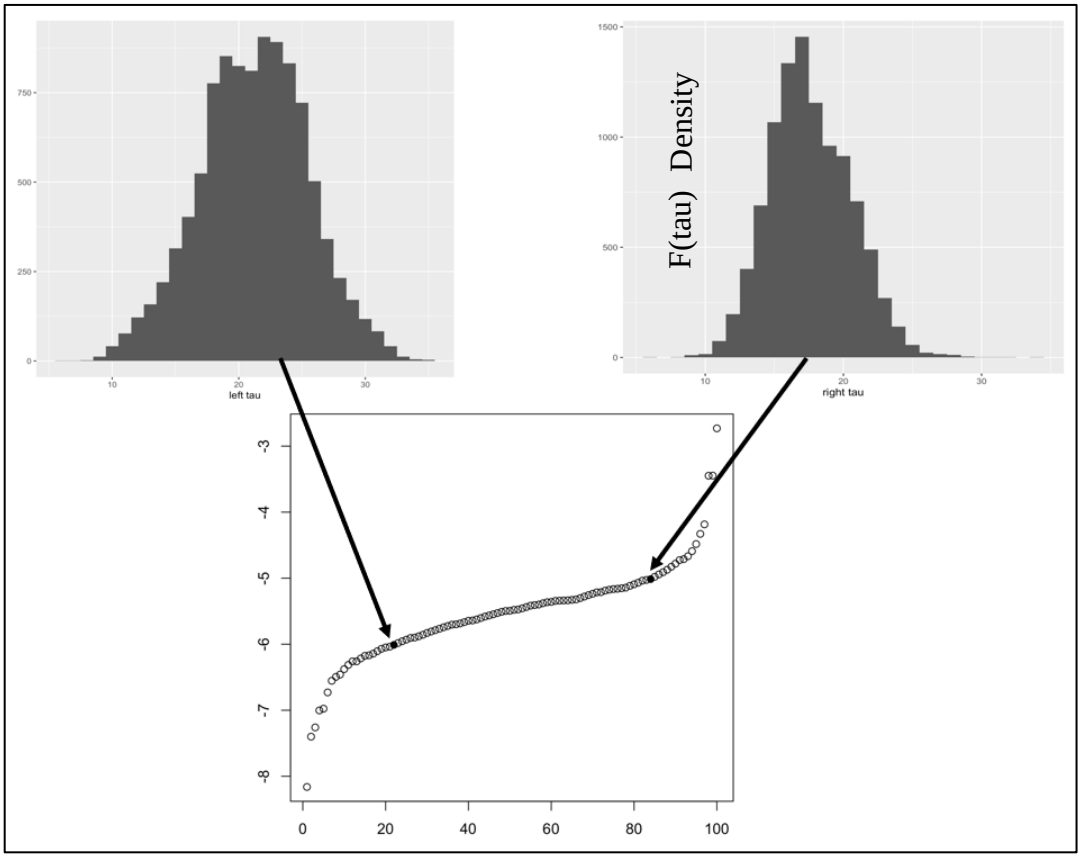

Figure 5: *Change point analysis for left-washed fingers. The two change-point marked in 100 simulated sorted donor plot (bottom) based on the highest probability from the left and right tau distributions (top two graphs). n=100. The y-axes for the distribution graphs are the densities for most probable occurrence.*

Each change point indicates the highest probability for which category a donor would fall under based on the log concentrations for all sampling locations and collections. As discussed, washed finger data were deemed the most suitable sample type for this analysis. Table 6 shows four examples for shedder status predictions for the listed four donors based on the RW collections. Donor 32 shows to have a very high probability of being a high shedder for collections 1 and 3 (Pr (hi)), with percentage above 99 for their RW location. On the second collection, the highest probability the donor falls under is medium shedder, with a probability of 81.4% (Pr (me)) and only 17.7% that the donor is a high shedder. Donor 39 has a higher probability to be a low shedder than high shedder because both of their first and last collections have a percentage of higher than 60% for low (Pr (lo)). Interestingly, the second collection is very close to the 50%, nearly split between low and medium. Donor 45 is considered to be a low shedder since all three of the collections for RW are more probable of placing them in the low category. One could also state that the chances that donor 45 is a high shedder is low since the probability to be a high shedder ranges only from 2-3.2% for the three collections, while the probability for the low shedding category is 52.8%. 71.1%, and 69%. Donor 50 is an example of a consistent prediction of a medium shedder since both two of the collection days had a medium shedder probability percentage of above 90%, with 76.4% for the second week collection.

Table 6: Prediction example for the most likely category of shedding propensity for the right washed finger location

RW						
Donor	Collection	log(amount.DNA)	Pr(lo)	Pr(med)	Pr(hi)	Most.Prob.Shed.Status
32	1	-2.0306038	0	0.6	99.4	High
32	2	-4.5693114	0.8	81.4	17.7	Med
32	3	-1.4202512	0	0.2	99.7	High
39	1	-7.0097891	68.8	29.2	2	Low
39	2	-6.4727391	42.5	55.2	2.3	Med
39	3	-6.8542467	63.6	34.4	2	Low
45	1	-6.6393027	52.8	45.1	2.1	Low
45	2	-7.3905524	71.1	25.7	3.2	Low
45	3	-7.0190411	69	29	2	Low
50	1	-5.0605581	1.9	90.6	7.6	Med
50	2	-4.4322216	0.7	76.4	22.9	Med
50	3	-5.026151	1.8	90.3	8	Med

The highest probability values for each donor per collection were used to assign a “low”, “medium” or “high” shedding propensity or shedder status based on several sample types. Table 7 shows some of the examples of categorizing some of the donors with locations RW and arm. Over the three collections, the probabilities of donors 42 and 45 are consistent across the all three collections and all three sampling locations; both were either medium (orange) or low (peach). Donor 32 is in between medium and high, shown in colors orange and blue; over the three collections, the most-probable-shedder-status changes across the sampling locations, but looking at only washed fingers, most of the predictions are for high shedder. This is different for donor 48, where the most-probable-shedder-status changes between left and right fingers, but is consistent over the three collection days when focusing on one location. There are some examples where donors fall under three different categories over the three collections and locations, this can be

observed in table 7 for donors 37 and 49. Table 7 illustrates the problem of assigning a shedding propensity based on a single sample type and single collection event.

Table 7: Prediction examples for the most probable shedder status** for three sample types Explain the color coding in the table descriptions.

			LW	RW	A
Donor	Collection	Gender	Most.Prob.Shed.Status	Most.Prob.Shed.Status	Most.Prob.Shed.Status
32	1	F	Med	High	High
32	2	F	High	Med	Med
32	3	F	High	High	Med
42	1	F	Med	Med	Med
42	2	F	Med	Med	Med
42	3	F	Med	Med	Med
45	1	M	Low	Low	Low
45	2	M	Low	Low	Low
45	3	M	Low	Low	Low
37	1	F	Med	Low	Med
37	2	F	Med	Med	Med
37	3	F	High	Med	Med
48	1	F	Med	Low	Med
48	2	F	Med	Low	Med
48	3	F	Med	Low	Med
49	1	F	High	Med	Med
49	2	F	High	Med	Low
49	3	F	High	High	Med

** The table lists separate results for each collection event; a category was assigned if the prediction model assigned a shedder status probability of 50% or more.

Discussion

An ideal skin sampling location to determine shedder status should be a site that minimizes self-DNA from other parts of the body, is able yield a DNA amount that is correlated with the DNA shed from washed fingers, and yields a constant amount of DNA over the three collection days. Tobias et al. (2017) concluded that there was no significant difference of DNA amount collected from both unwashed and washed hands

over their three-non-consecutive collections. Contrary to these results, there was a high variance between three-non-consecutive collections of the unwashed finger tape-lifts per donor observed in this study. The washed finger locations, instead, showed a smaller variance over the three collections per donor compared to the unwashed in this study. Agreeing with Lowe et al. (2002) and Phipps and Petricevic (2007), a significant difference of DNA amount was observed between unwashed and washed fingers, and a larger amount of DNA was observed in the unwashed fingers as compared to the washed fingers. This differs from results obtained by Goray et al. (2016), who concluded that there was no significant difference in DNA amounts between these two sets. All three of these studies looked at shedding propensity by testing unwashed or washed palmar skin, while our work included sebaceous skin as well.

The ear location samples showed the highest DNA concentration and a low number of mixtures, indicating that ear sampling location could be a great source to collect self-DNA (Trapani, 2021). However, despite its high source of self-DNA, it is not a suitable location to determine the shedder propensity due to a low correlation with samples collected from fingers. Additionally, its high proportion of variation from day to day shows that shedding propensity cannot be represented by one collection, and the low variation between individuals would make it difficult to distinguish donors by their shedding propensity based on the ear samples. It is possible that the high amount of DNA and the inconsistency over collections might be due to different daily hygiene routines.

Although arm sampling locations showed a higher correlation with some palmar locations compared to other sebaceous locations, its daily variation between the three collections was higher than for the palmar skin locations such as the washed fingers; this

indicates that washed finger collection sites yield a more consistent result over the collections than the sebaceous sampling locations. The arm sampling locations showed a relatively lower variation within the donor group. Again, these factors are preventing arm samples to be suitable for shedding propensity determination. Nape sampling locations provided a higher amount of DNA than the arm sampling locations. Nape sample variation observations were similar to arm samples, except the arm had a higher correlation with the washed palmar skin results. Due to their lack of correlation with the palmar locations, these sebaceous skin locations are not suitable candidates to test for shedder propensity.

In comparison to the nape and ear sample collections, washed finger samples yielded a much lower DNA concentration. This was different for unwashed friction-ridge samplings. While the unwashed finger samples on average showed a lower DNA amount than the ear samples, there are some exceptions with some extremely high values in unwashed samplings that might be caused by the volunteers having had contact with a body fluid like saliva. Research has shown that aside from anucleate skin cells, nuclear DNA containing materials from other parts of the body, like body fluids and sebaceous secretions can be found on hands (Lacerenza et al., 2016, Zoppis et al., 2014). This explains the high values from the unwashed samples may be due to the DNA from other parts of the donor, as well as foreign DNA from touches or contacts from objects or people.

Unlike the low correlations observed for sebaceous samplings, washed palmar sampling locations showed a strong correlation with the unwashed finger sampling locations. Washed palmar sampling locations showed a higher correlation with the

unwashed palmar samples and a high variation between donors as well as low variation across the daily collections. This finding of the low variance between the sample collections did not agree with Oleiwi et al. (2015), who showed a difference in relative shedding propensity for their six volunteers over four collections for washed hands. The washed finger pair gave the highest correlation out of all other combinations, which is not surprising since both left and right hand samples were collected at the same time under the same conditions.

To determine shedding propensity, the sample collected from the sampling location must be representable with one time collection and show high inter-individual variability to categorize donors. Washed palmar sampling locations are the most desirable collection site to determine shedding propensity compared to other collections sites. First would be the right washed fingers, which had the lowest three-day variation and the highest donor variation of all sample locations as shown in table 5; second would be the left washed fingers. These finger locations also have a low variation between collections, this consistency would allow for tape samples to be collected once to determine the shedding propensity. Furthermore, the high variation between donors indicate that it would be possible to categorize shedding propensity for all individuals in a population.

The toe is an undesirable sampling location to determine shedding propensity. Even though the toe surface is also palmar skin, there was a lack of correlation to the fingers. With inconsistent results over three days there is only a low possibility to categorize shedding propensity with one collection, and this sample type had low variation between individual donors. Additionally, it would be complicated to compel uncooperating donors to take off socks and shoes. Although the toe is also a friction ridge

surface and the sample collection process did not include washing prior to collection, the location provided the least amount of DNA compared to all other sample types, including unwashed fingers. Socks and other objects in contact with the toe might play a role in the low concentration detected and the divergent results over the three collections.

In addition to the exploration of different sample types, the data was used to generate a prediction model to predict the shedder status of an individual using Bayesian statistics; examples of the process for the model are shown in Figures 4 and 5. This prediction model associated DNA concentrations with a specific class of shedder (low, medium and high) and thus would categorize shedder propensity based on the highest probability obtained from a given log transformed DNA concentration for each sampling location. This was further explored for the washed finger and arm samples. Some of the probabilities in the prediction model fluctuate over the three collections and these shedder statuses of the donors were mostly scored in between two categories. The prediction model showed that there are more medium shedders observed than high or low, something that has been found by other researchers as well, for example Daly et al. (2012) and Manoli et al. (2016).

Several individuals had results in multiple categories, some of them are between high and medium, and some are low and medium. Splitting shedding propensity into more than three categories may help mitigate the ambiguous divisions of this inconclusive category. For example, volunteers who scored a high probability in both the medium and the high for one sampling location over the three collection days could be sorted under medium-high. Corrections and improvements could be explored in future research to edit the model for more consistent predictions. Shedding propensity is known

to change with age (Poetsch et al., 2013), but it is unknown which other factors play a role. Taylor et al. (2017) question if shedding propensity is really a biological characteristic or more based on personal habits like handwashing, or nail biting. But the same group conceded that some individuals consistently deposit more DNA than others (Taylor et al., 2016). Maybe individuals with a high shedding propensity maintain this trait over time while the propensity for medium shedders could be more prone to change.

Conclusions

This study explored the potential of using sebaceous sampling locations to determine shedding propensity, something that had not been studied in previous studies. Past shedder status studies focused on palmar sampling locations, and they would categorize an individual in a low medium or high shedder category based on DNA concentration and profile quality (Lowe et al., 2002, Allen et al., 2008, and Farmen et al., 2008). We also investigated how different sebaceous sampling locations may be used to predict shedder status, but based on the results, only the washed finger sampling locations can be considered suitable.

An explanation for the lack of correlation between sebaceous skin and palmar skin, could be due to personal behaviors and hygiene, as well as natural biological factors. The presence of sebum is one of these factors. According to Van Den Berge et al. (2016), there is more DNA detected on sebaceous skin compared to palmar because of sebum. Personal behaviors such as face touching would increase the amount of DNA on hands that can be deposited on the surfaces, and this could contribute to the higher concentration of DNA found on the unwashed finger samples. This agrees with

Lacerenza et al. (2016) and Zoppis et al. (2014), who detected sebum components on palmar samples. If sebaceous skin is still being explored to be a standard collection site, further research could be on whether these factors have any effect on how much DNA could be collected from one time. A correction needs be applied to find the shedding propensity of a person, and these corrections may form from statistic of a new formula. Although sebaceous and palmar skin are not highly correlated, it is still necessary to understand the occurrence of high DNA amount on sebaceous skin. For example, when determining active or passive donors in mixture STR profiles obtained from surfaces of crime scenes, analysts would need to consider the possibility of sebaceous DNA contributing to the mixture profile.

The consistency of daily collection over the three-days for the washed finger samples with tape-lifts suggested that tape-lift allows a one-time sample collection to predict shedding propensity. The standard size of the D-Squame tape-discs ensures a uniform collection method. To improve on more precise prediction of shedding propensity, there are still factors that need to be investigated. In the continuation of this study, biological factors such as age, ethnicity, skin diseases, and life-style will be surveyed using tape-lift on the washed finger locations as the sampling location.

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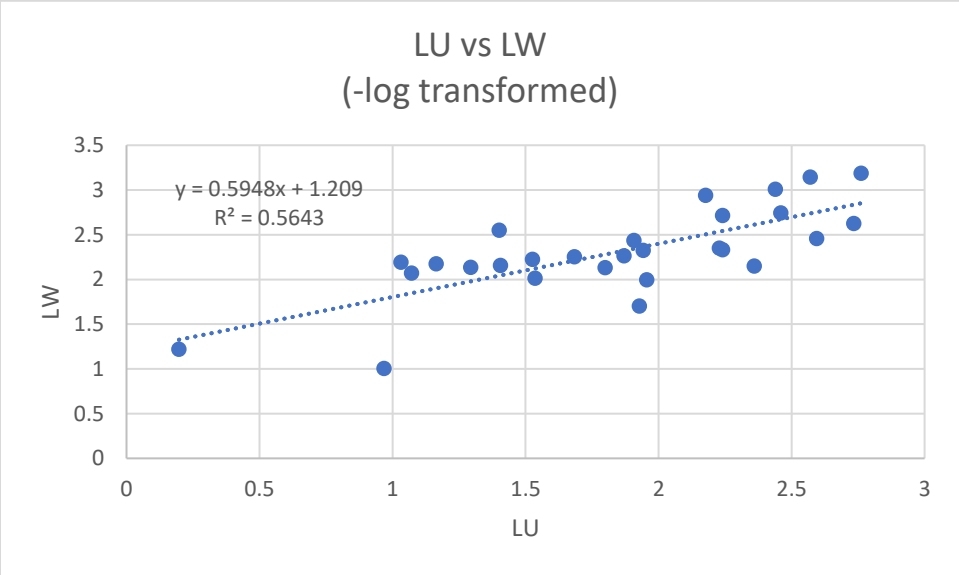
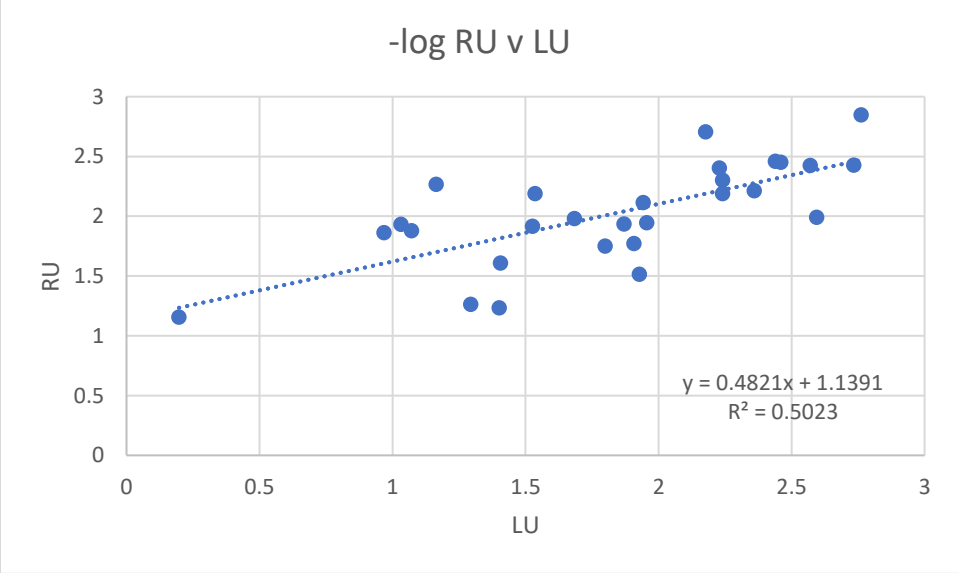
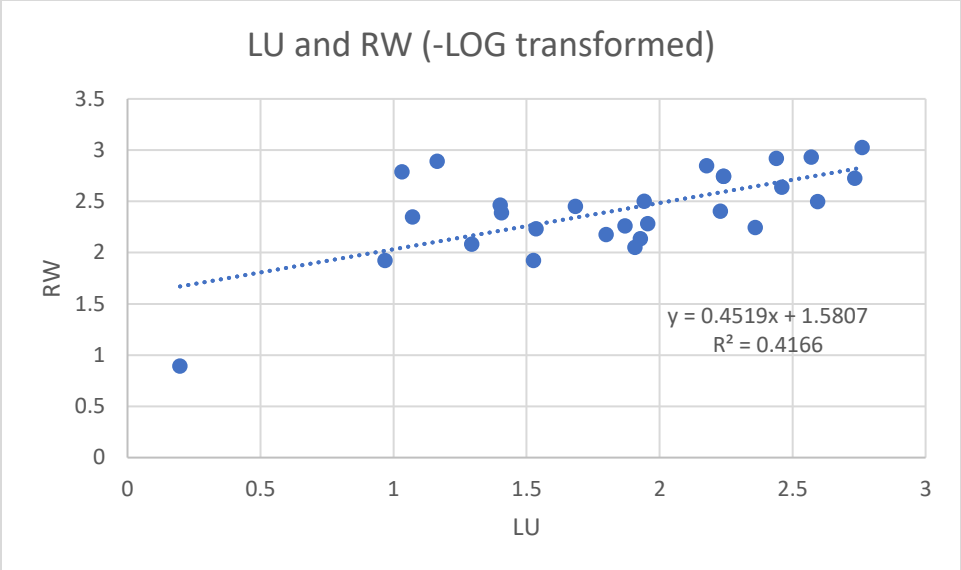
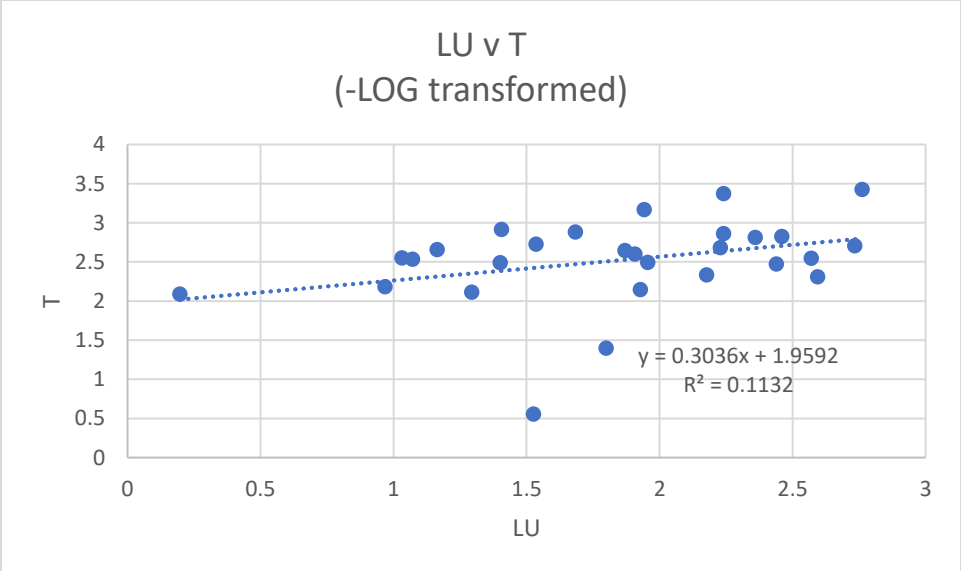
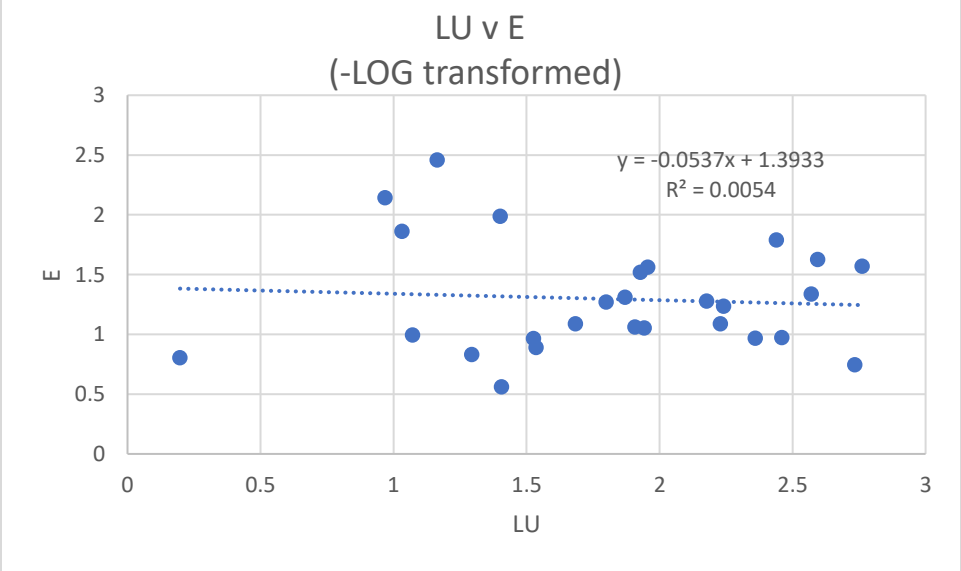
Appendix A – Correlation plots for DNA concentrations (average over three day collections) for all combinations of skin locations

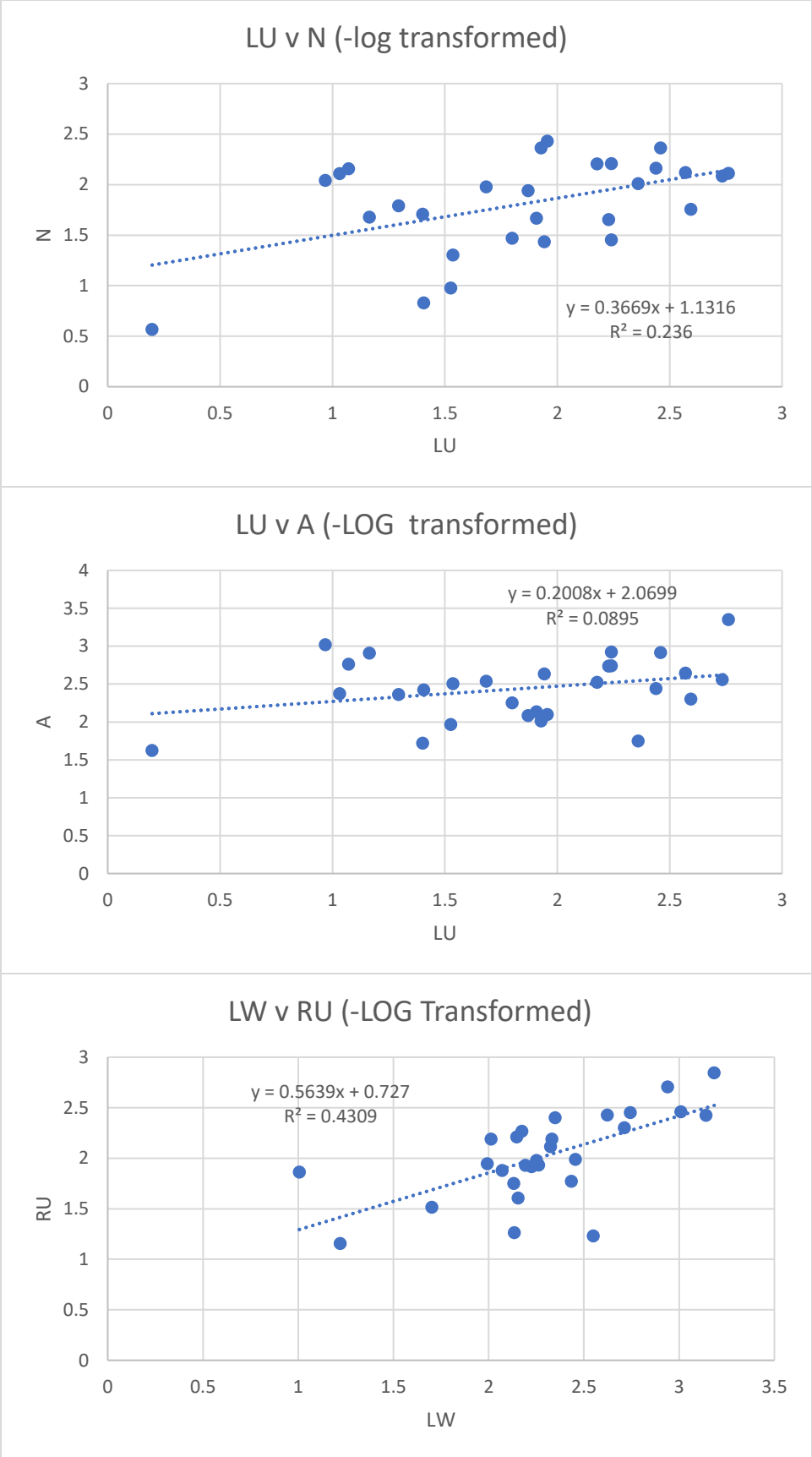


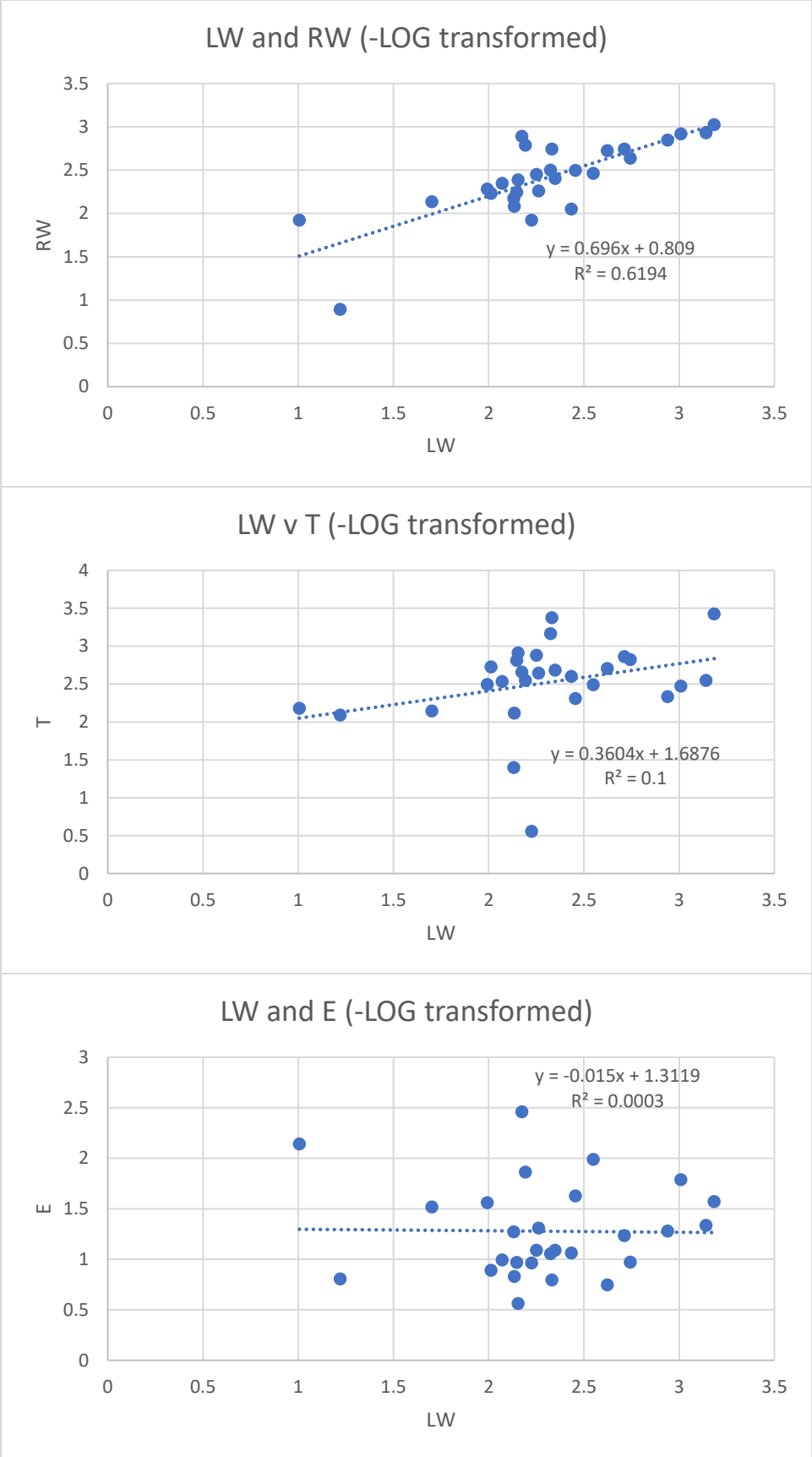


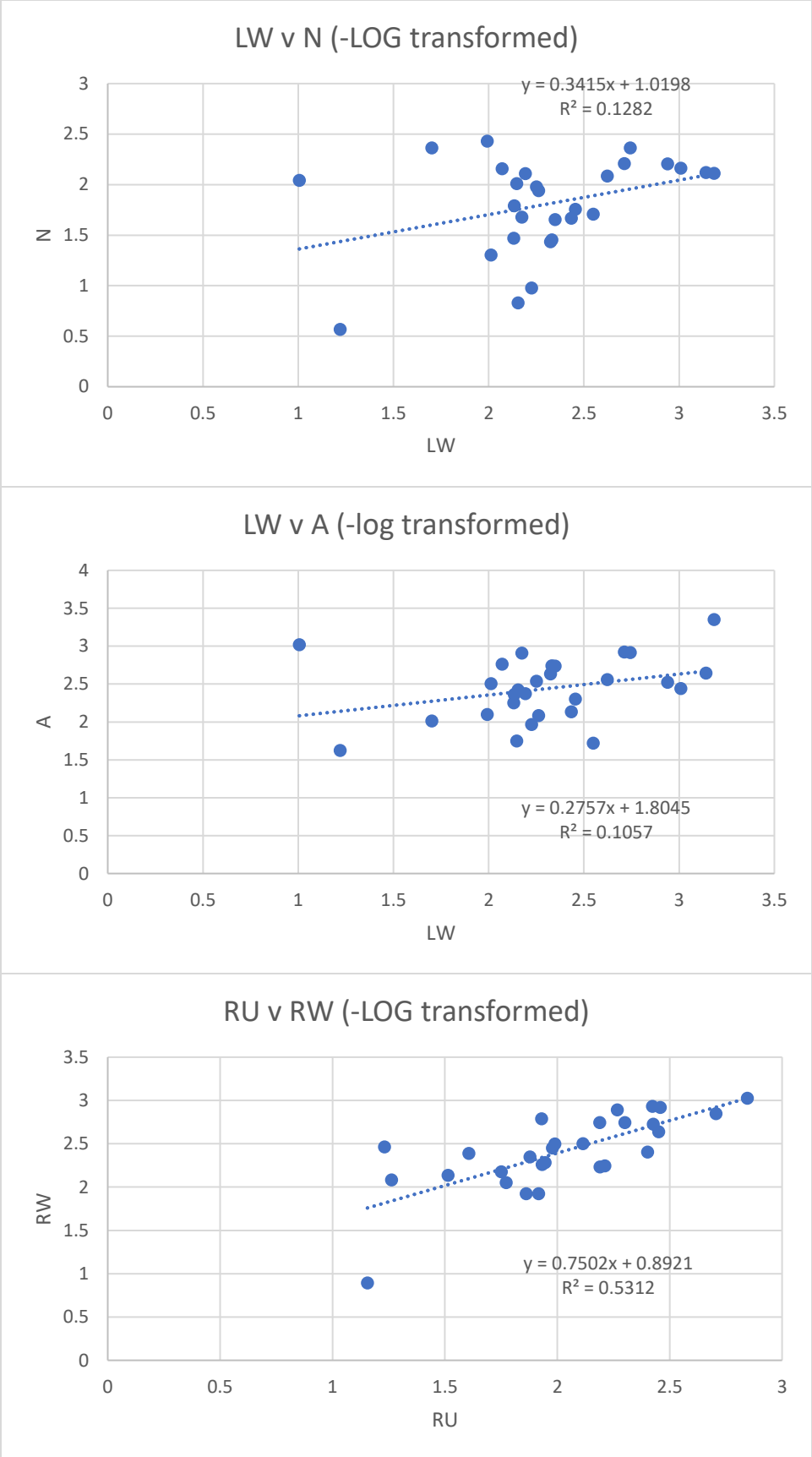

LU and RW (-LOG transformed)
RW
LU
y = 0.4519x + 1.5807
R² = 0.4166

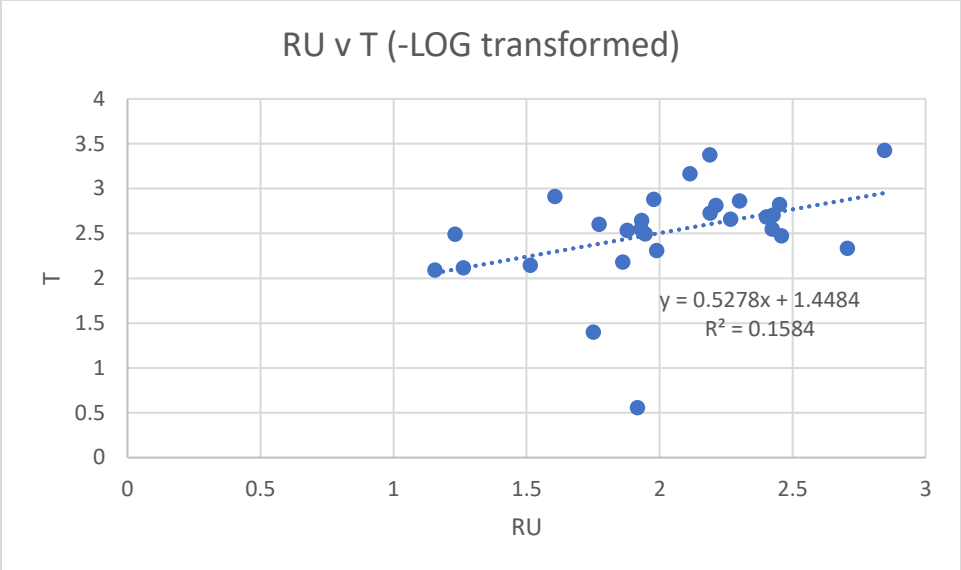

LU v T
(-LOG transformed)
T
LU
y = 0.3036x + 1.9592
R² = 0.1132

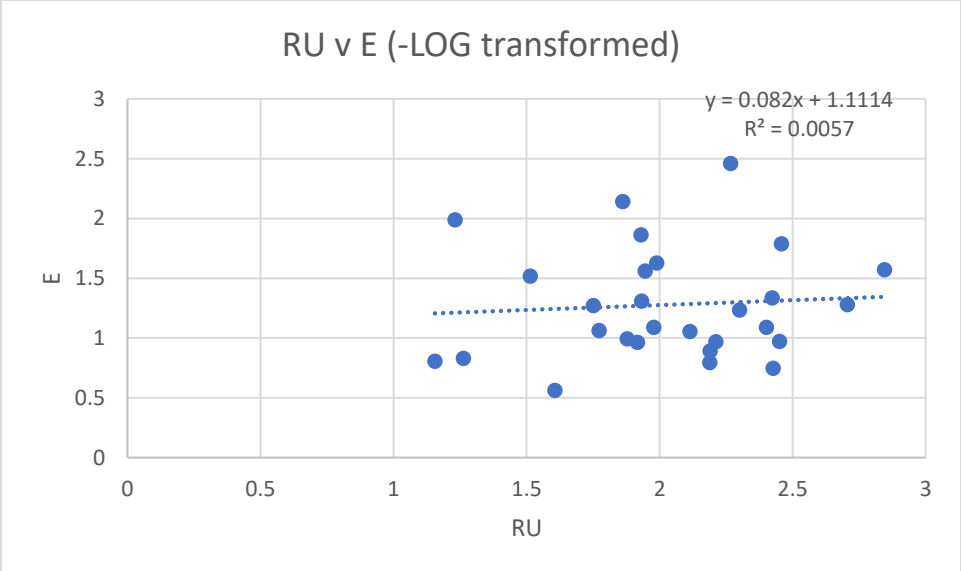

LU v E
(-LOG transformed)
E
LU
y = -0.0537x + 1.3933
R² = 0.0054

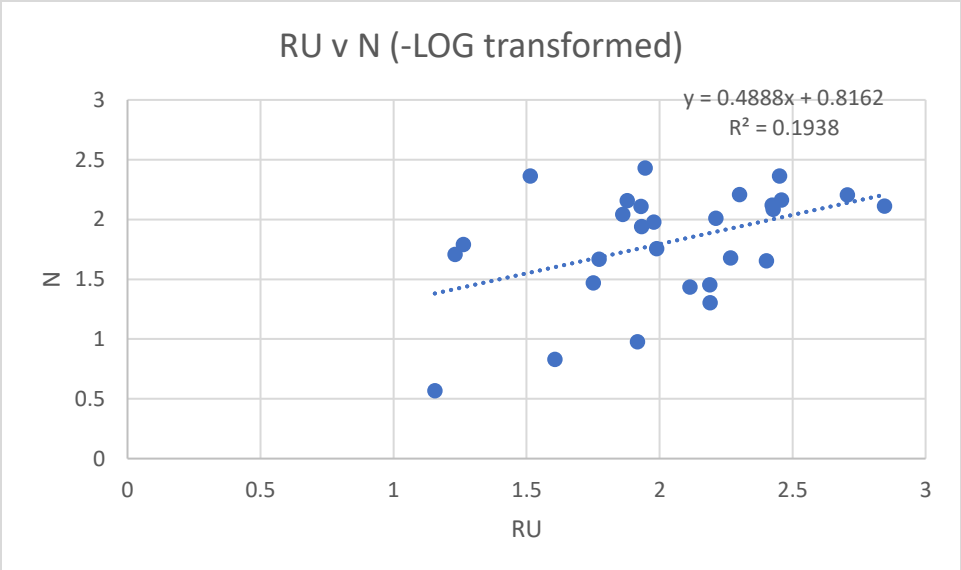

LU v N (-log transformed)
N
LU
y = 0.3669x + 1.1316
R² = 0.236
LU v A (-LOG transformed)
A
LU
y = 0.2008x + 2.0699
R² = 0.0895
LW v RU (-LOG Transformed)
RU
LW
y = 0.5639x + 0.727
R² = 0.4309

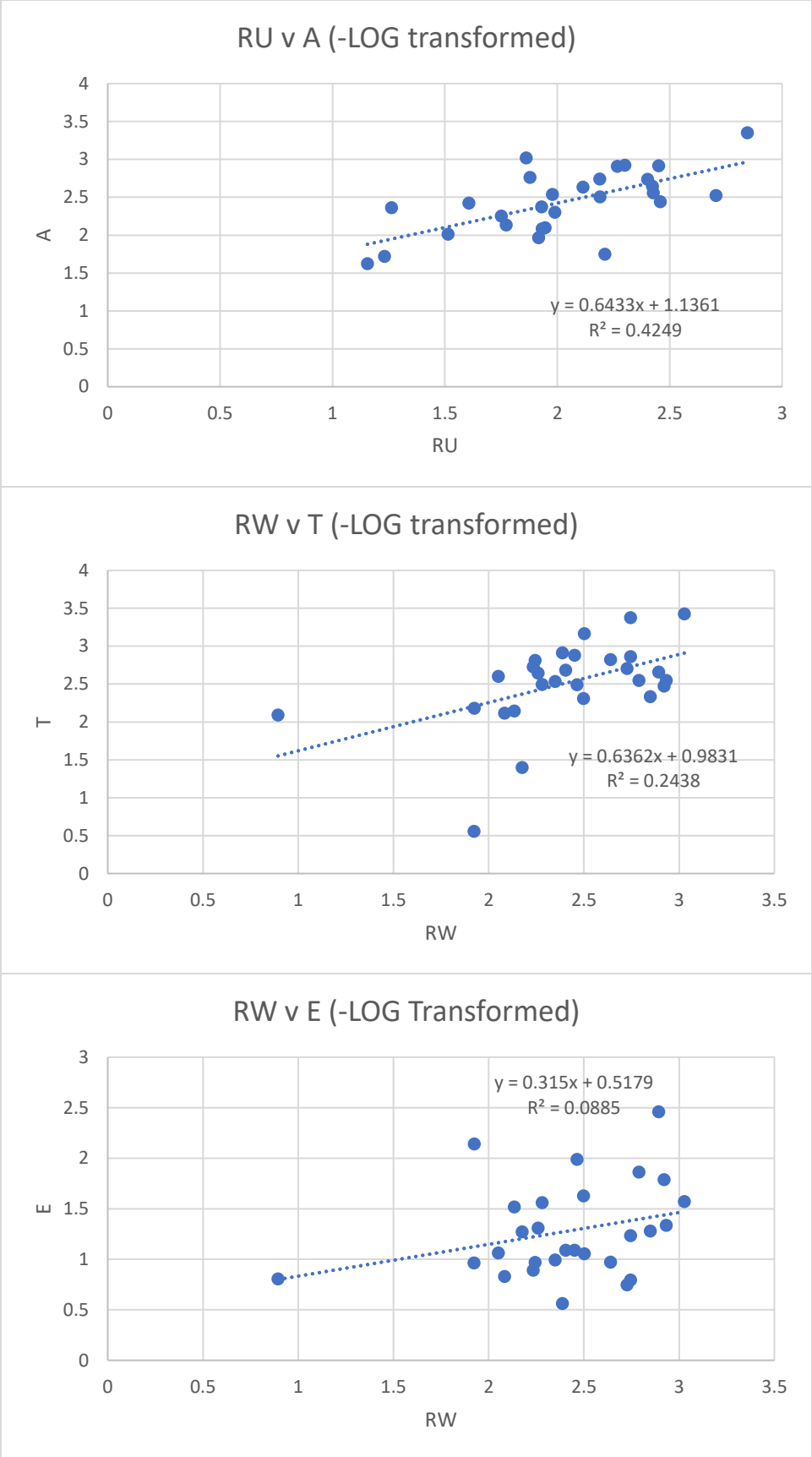

LW and RW (-LOG transformed)
RW
LW
y = 0.696x + 0.809
R² = 0.6194
LW v T (-LOG transformed)
T
LW
y = 0.3604x + 1.6876
R² = 0.1
LW and E (-LOG transformed)
E
LW
y = -0.015x + 1.3119
R² = 0.0003

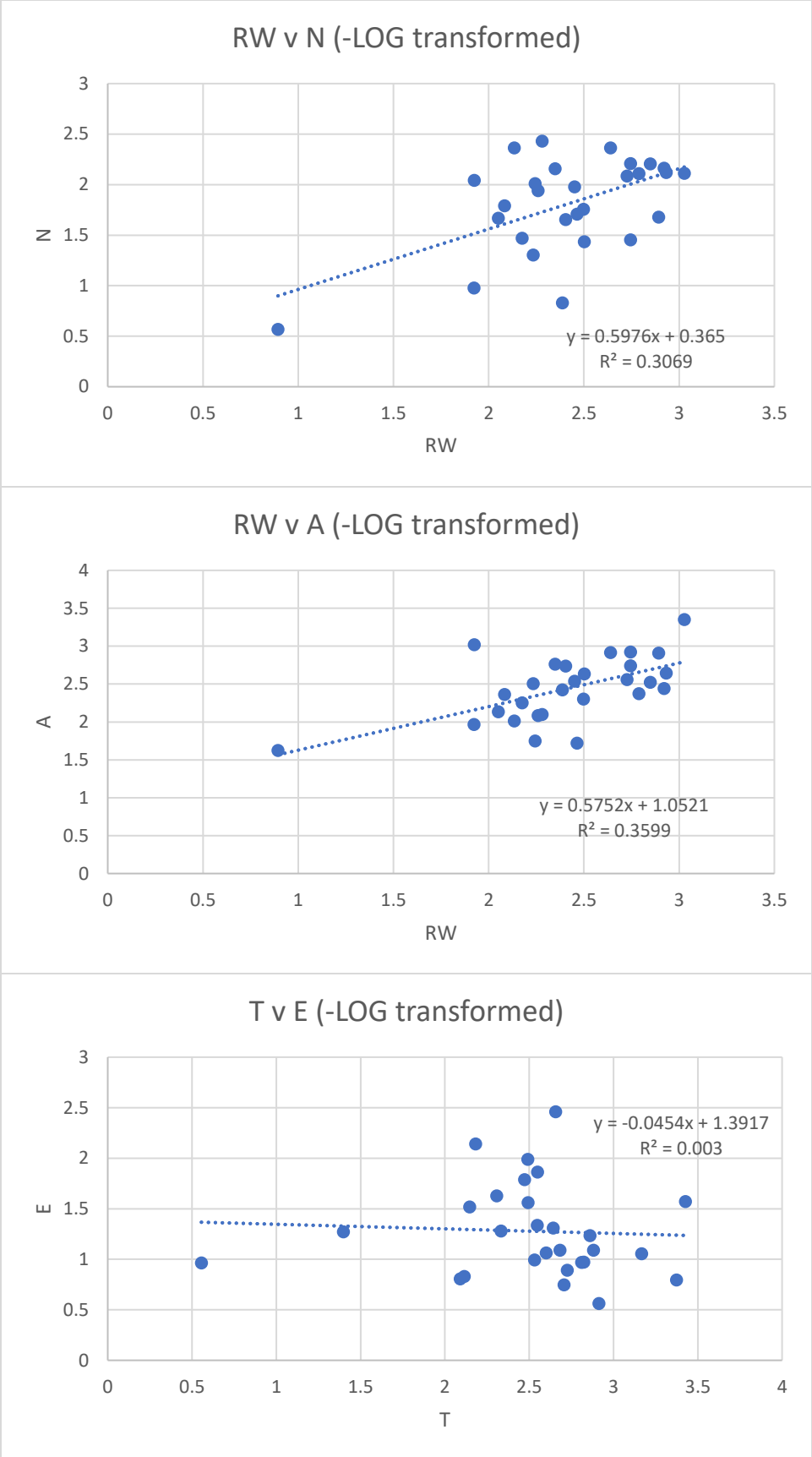

LW v N (-LOG transformed)
y = 0.3415x + 1.0198
R² = 0.1282
N
LW
LW v A (-log transformed)
y = 0.2757x + 1.8045
R² = 0.1057
A
LW
RU v RW (-LOG transformed)
y = 0.7502x + 0.8921
R² = 0.5312
RW
RU

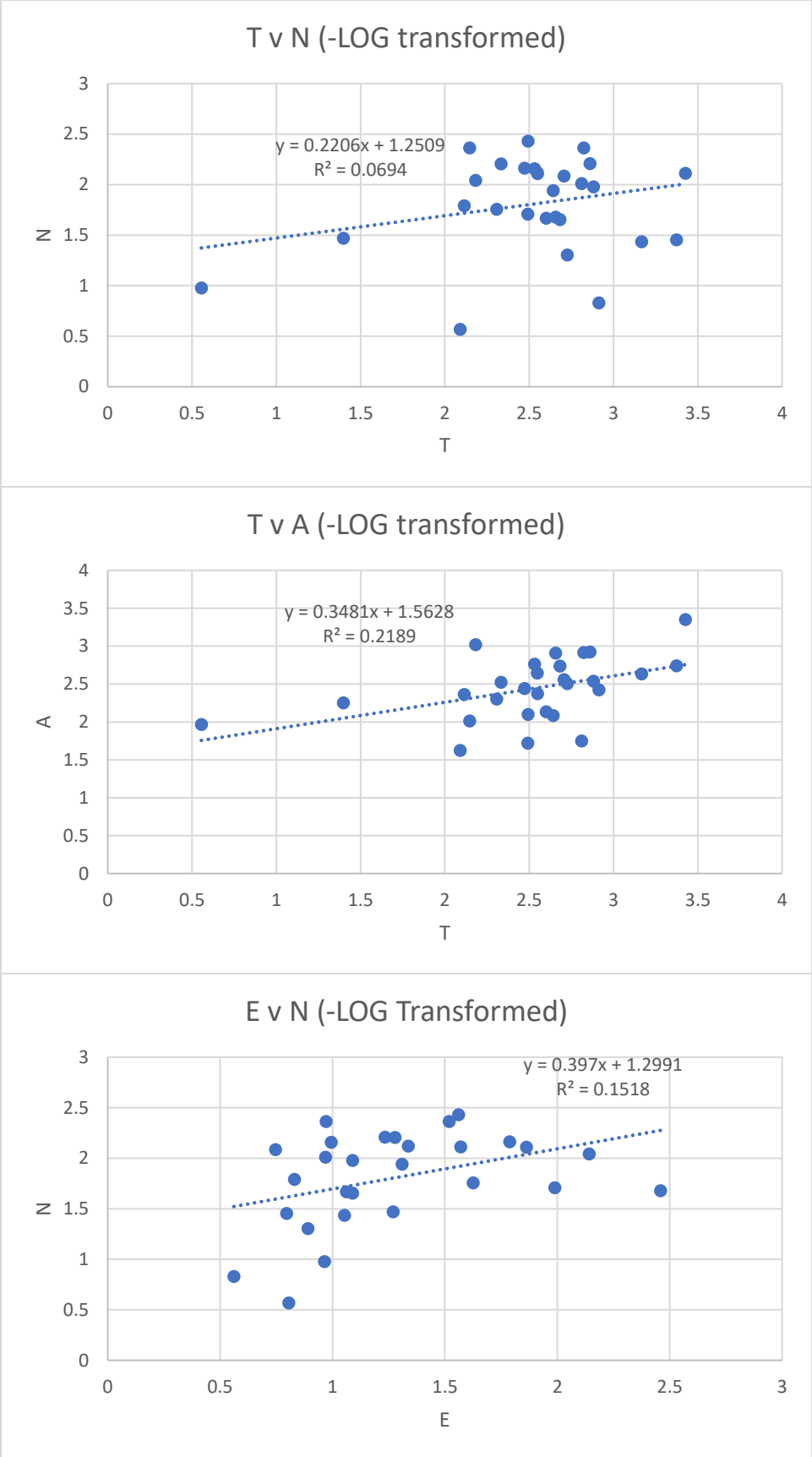

RU v T (-LOG transformed)
T
RU
y = 0.5278x + 1.4484
R² = 0.1584
0
0.5
1
1.5
2
2.5
3
3.5
4

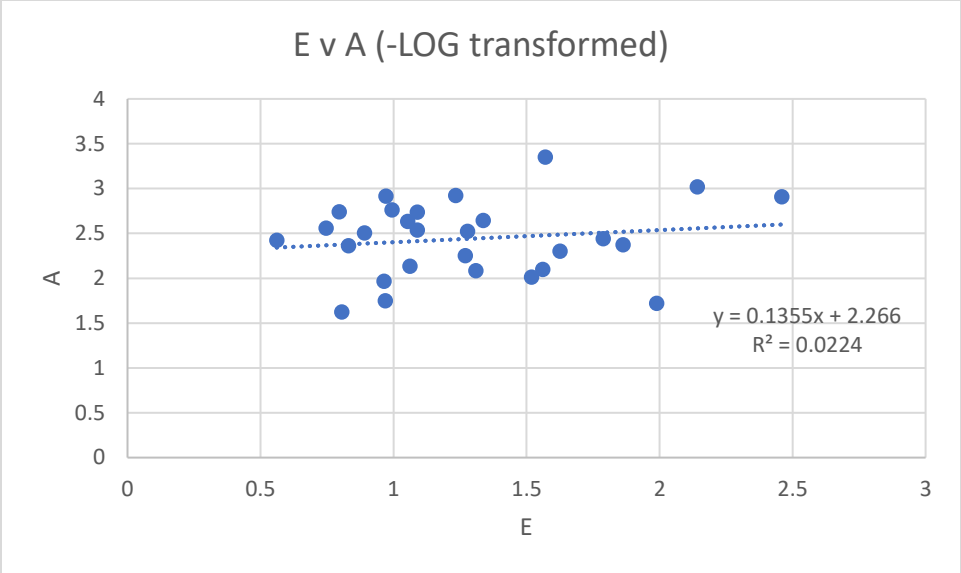

RU v E (-LOG transformed)
E
RU
y = 0.082x + 1.1114
R² = 0.0057
0
0.5
1
1.5
2
2.5
3

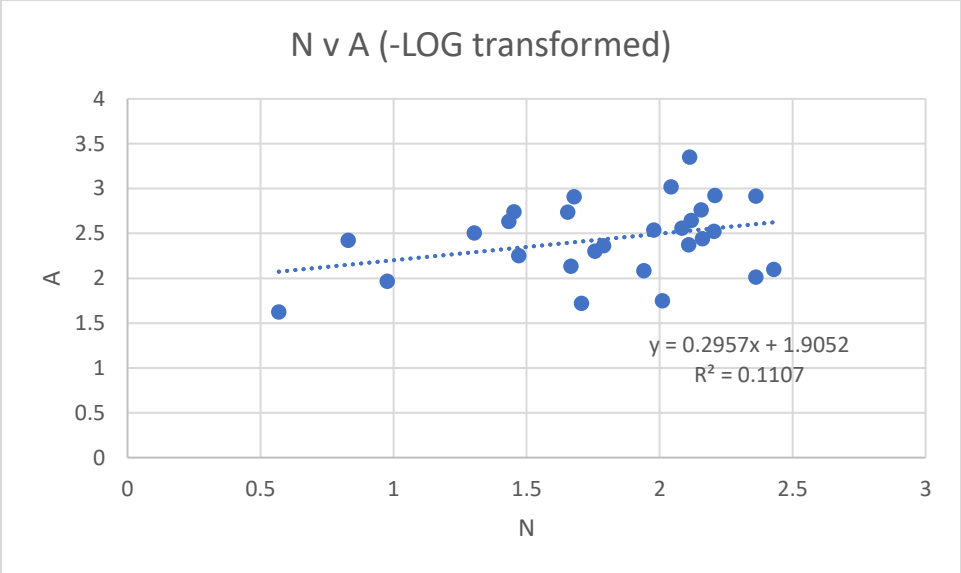

RU v N (-LOG transformed)
N
RU
y = 0.4888x + 0.8162
R² = 0.1938
0
0.5
1
1.5
2
2.5
3


RU v A (-LOG transformed)
A
RU
y = 0.6433x + 1.1361
R² = 0.4249
RW v T (-LOG transformed)
T
RW
y = 0.6362x + 0.9831
R² = 0.2438
RW v E (-LOG Transformed)
E
RW
y = 0.315x + 0.5179
R² = 0.0885


RW v N (-LOG transformed)
N
RW
y = 0.5976x + 0.365
R² = 0.3069
RW v A (-LOG transformed)
A
RW
y = 0.5752x + 1.0521
R² = 0.3599
T v E (-LOG transformed)
E
T
y = -0.0454x + 1.3917
R² = 0.003


T v N (-LOG transformed)
y = 0.2206x + 1.2509
R² = 0.0694
N
T
T v A (-LOG transformed)
y = 0.3481x + 1.5628
R² = 0.2189
A
T
E v N (-LOG Transformed)
y = 0.397x + 1.2991
R² = 0.1518
N
E


E v A (-LOG transformed)
A
E
y = 0.1355x + 2.266
R² = 0.0224
0
0.5
1
1.5
2
2.5
3
3.5
4


N v A (-LOG transformed)
A
N
y = 0.2957x + 1.9052
R² = 0.1107
0
0.5
1
1.5
2
2.5
3
3.5
4