



## Human background DNA on stones in an urban environment

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### ABSTRACT

Stones are frequently used as tools in criminal acts. In our department, around 5 % of all analysed crime scene related trace samples are contact or touch DNA traces swabbed from stones. These samples are primarily related to cases of damage to property and burglary. In court, questions can arise about DNA transfer and the persistence of background DNA not related to the respective crime. To shed some light on the question of how likely it is to detect human DNA as background DNA on stones from an urban environment, the surfaces of 108 stones sampled throughout the city of Bern, the Swiss capital, were swabbed. We detected a median quantity of 33 pg on the sampled stones. STR-profiles suitable for a CODIS (Combined DNA Index System) registration in the Swiss DNA database were established from 6.5 % of all sampled stone surfaces. For comparison, retrospective casework data analysis from routine crime scene samples demonstrates a success rate of 20.6 % for the establishment of CODIS-suitable DNA profiles from stones sampled for touch DNA. We further investigated how climatic conditions, location and properties of the stones affected the quantity and quality of the recovered DNA. In this study, we show that the quantity of the measurable DNA decreases significantly with increasing temperature. Furthermore, less DNA could be recovered from porous stones, compared to smooth ones.

### 1. Introduction

DNA trace analysis plays an important role in crime investigations as material evidence. Only a few cells or DNA copies recovered from a touched object are sufficient to establish a DNA profile [1], inevitably raising questions about the origin of such minute amounts of biological material [2,3]. Stones are relatively common tools in burglaries or property damage and are frequently used as thrown objects during riots. Following the establishment of the DNA profile from a stone that was supposedly involved in an offense, a suspect will at least be confronted to the question of why his or her DNA was found on a stone at the site of the crime. Concerns about background DNA and its persistence challenge the probative force of DNA profiles generated from stones that were either found in a public environment or picked up in such an environment before the act. Therefore, we intended to investigate whether DNA can be recovered from stones found in public spaces, i.e. non-crime scene related locations. This DNA, present on stones without known history of use, is in the following referred to as background DNA, conforming to the definition of van Oorschot et al. [2]: "The DNA present on the surface prior to the deposit of interest being placed on the surface

during the action of interest."

Most traces collected from stones are reported as contact traces. According to Alketbi [4], touch DNA or contact trace DNA refers to "the transfer of DNA through skin cells when an object is either touched or handled". However, *touch* or *contact* defines the type of the transfer, which is not known for a trace found at a crime scene. In most cases, no pre-tests are performed to determine the origin of the biological material having been sampled. Although the police indicate most DNA traces as contact traces, it is not certain that the trace was left by contact or by deposition of secretion.

In this study, the surfaces of 108 stones from the public urban area in Bern, Switzerland, were analysed for the presence and quantity of DNA. The generated STR profiles were classified and their suitability for a database comparison as a single or major profile assessed. In addition, we evaluated whether there is a correlation between the detected DNA quantities and qualities with the location, the surface roughness of the stones and the weather data of the past three days. The experimental data was compared to casework data of the Forensic Molecular Biology Department in Bern.

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## 2. Methods and materials

### 2.1. Sampling

The sampling was performed in the city of Bern, Switzerland, between March and June 2022. A total of 108 stones were sampled on 9 non-consecutive days. Our selection criterion was a length of at least 6 cm for the longest diameter in a lying position, for details see [Supplements, Table 1](#). All stones were swabbed on site with a pre-moistened viscose forensic swab (Sarstedt, Nümbrecht, Germany) and weighed. Four stones (8, 32, 76, 90) were swabbed with two swabs, as the first one was frayed before all sides were swabbed. Since more DNA is expected to be found on the upper side rather than the one facing the ground, and to avoid contamination or abrasion, the stones were swabbed without moving, i.e. only the visible surfaces. Size measurement was done relative to the standard on the taken picture, using the imaging App Image Meter (Dirk Farin – Algorithmic Research, Stuttgart, Germany).

### 2.2. Sampling conditions and evaluation

The porosity, the cleanliness, the sky state, the roofing and the potential for background DNA were determined subjectively (see [Supplements, Table 3](#)).

"Sky state" describes the cloud coverage or the solar radiation while sampling. If the stone was in a place that is covered, this is reflected by the variable "roofing". The potential for background DNA was determined subjectively, depending on the location in the city and the number of people in the surrounding area.

To see if there is a correlation between the weather and the DNA quantity or the degradation detected, the weather during the sampling period was recorded for the temperature (°C) [5], the humidity (%) [5] and the rainfall per day ( $L\ m^{-2}$ ) [6] (see [Supplements, Table 3](#)). The minimum and maximum value of a day was recorded for temperature and humidity, and the average built. The average values of the three days before the sampling day were used for the statistical evaluation.

### 2.3. Analysis

The analysis was conducted using the standard operating procedure for contact traces of the Forensic Molecular Biology Department of the Institute of Forensic Medicine Bern.

DNA from swabs was extracted using the PrepFiler Express™ Kit (Thermo Fisher, Waltham, MA, USA). 500  $\mu$ L lysis buffer and 5  $\mu$ L 1 M Dithiothreitol (Sigma-Aldrich, St. Louis, MO, USA) were added to the tube with the swab head. After two times 30 s at 5900 rpm on a Precellys® 24 Touch homogenizer (Bertin instruments, Montigny-le-Bretonneux, France), the samples were incubated at 56 °C overnight on a thermo-shaker at 400 rpm (Labgene Scientific SA, Châtel-Saint-Denis, Switzerland). Purification was performed with the AutoMate Express™ Nucleic Acid DNA Extraction System (Thermo Fisher, Waltham, MA, USA) with an elution volume of 50  $\mu$ L.

The quantification of the extracted DNA was done by quantitative PCR using the Quantifiler™ HP Kit on a 7500 Real-Time PCR System with the HID Real-Time PCR Analysis Software v1.2 (Thermo Fisher, Waltham, MA, USA). Not only the quantity but also the degradation index was measured [7].

By using multiplex-PCR, DNA was amplified in 25  $\mu$ L reaction volume using the AmpFLSTR™ NGM Select™ Kit (Thermo Fisher, Waltham, MA, USA) on a T3000 Biometra Thermocycler (Analytik Jena, Jena, Germany). All samples with a DNA concentration below 20  $\mu$ L<sup>-1</sup> were amplified with 32 cycles instead of 30 cycles as default. The desired DNA input for multiplex PCR is 0.5 ng. If samples are less concentrated than 0.05 ng  $\mu$ L<sup>-1</sup> we used the maximum sample volume of 10  $\mu$ L. All samples were amplified by default, even if quantification detected no DNA.

Capillary electrophoresis was run on a 3500 xL genetic analyser with

the 3500 Series Data Collection Software v3 (Thermo Fisher, Waltham, MA, USA). The following signal interpretation was done with GeneMapper™ ID-X v1.6 (Thermo Fisher, Waltham, MA, USA). All peaks above 100 rfu were considered as true alleles. Minimum number of contributors (NOC) were determined with the maximum allele count method (MAC), with all amplifications of the respective sample considered.

### 2.4. Criteria for submission to the Swiss CODIS Database

Regulated by law, Switzerland has defined the 16 STR loci, amplified by the AmpFLSTR™ NGM Select™ Kit, as database loci [8]. The Swiss DNA database uses the CODIS software to compare newly established DNA profiles with registered profiles. For single or major profiles, the minimum entry criteria are six loci and eight loci for two-person mixtures [9]. In this study, only single or major component profiles were considered. The sex locus Amelogenin does not count for the database criteria, because although it can be added, it will not be searched for [9]. To consider loci of a major profile as reliably typed, we defined in accordance with the recommendations from the German Stain Commission that a minimum peak-height-ratio of 4:1 for major to minor components must be fulfilled [10] and according to our lab internal standards, the peak height balance for heterozygous loci must be at least 60 %. In addition, the Swiss law prescribes that the loci have to be confirmed by at least a second amplification [8]. The obtained profiles were classified as "no profile", "not interpretable" and "CODIS suitable". The classification "no profile" includes the profiles with less than six double-determined loci and signals detected at less than half of the loci. The classification "not interpretable" includes the ones that do not fulfil the CODIS criteria e.g., because of peak height imbalances or a complex mixture profile. However, they might be interpretable by probabilistic genotyping, if reference samples were available.

### 2.5. Statistical evaluation

Statistical analyses were performed using R version 4.2.2 together with RStudio software v2022.12.0 + 353 [11] and the following packages: *dplyr*, *tidyr*, *ggplot2*, *tidymodels*, *broom.mixed* and *glmmTMB*. The analysis of the dependency of the variables *porosity*, *cleanliness*, *weight*, *sky state*, *roofing*, *potential for background DNA*, *temperature*, *humidity* and *rainfall* on the DNA quantity was done by fitting a zero-inflated linear model (R function *glmmTMB*), as we observed a clear enrichment in values below the limit of detection. To better approximate a normal distribution, we used the natural logarithm of the DNA quantities. As the presence of values below the limit-of-detection (LOD) of the quantification instrument would bias the mean and standard deviation of the observed DNA quantity distribution, we replaced zeros by half the LOD (i.e. 0.0025 ng). Values were then shifted left by subtracting the half-LOD-value in order to align the peak to zero and fit a zero-inflated model. To predict the degradation index with the variables, we fitted a simple linear model and computed regression coefficients, together with 95 % confidence intervals and p-values were computed using a Wald z-distribution approximation.

## 3. Results

### 3.1. Retrospective casework data analysis

From January 1st 2015 until March 8th 2022, 85.5 % of the registered traces in the Department of Forensic Molecular Biology were contact traces. From these contact traces, 5.3 % (n = 1527) were sampled from stones, mainly collected by swabs. It is not known if the traces indeed originated from skin contact, but the police indicated them as contact traces.

On average, 0.653 ng DNA per stone was detected, with the highest quantity being 56.1 ng. DNA could be detected on 95.1 % of the samples.

Sixty-five percent of the traces (992) contain up to 0.25 ng DNA, including 75 samples being "undetermined" in the qPCR, thus explaining the low median of 0.15 ng. From the 1527 samples, 314 profiles fulfilled the CODIS entry criteria for single/major profiles (20.6 %).

### 3.2. Distribution of sampled stones

The sampling was conducted in the old town of Bern and the neighbouring districts. The mapping of the locations show the distribution in the city (Fig. 1). Stones, suitable for throwing with the goal to cause serious damage, were not abundant in the city. To find twelve stones on one day, the searching time was three and a half hours on average, including sampling time. Sampling around the old city centre seemed particularly important to us, because this is where most political demonstrations take place, occasionally accompanied by riots.

### 3.3. DNA quantities and CODIS suitability

All raw data for the city stones can be looked up in the [Supplements, Table 3](#).

Human DNA could be detected on 69 % of the sampled stones. DNA quantifications ranged from 0 ng to 3.48 ng with a median of 0.033 ng and an average of 0.181 ng (Fig. 2a). Three stones were found painted due to an art project ([Supplements, Table 1](#), S29, S30, S31). No noticeably larger DNA amounts were detected on those painted stones and the profiles were not CODIS suitable, which is why they were not excluded from the analysis.

Out of 108 DNA profiles, seven profiles were suitable for submission to the CODIS database as a single or major component. This corresponds to a success rate of 6.5 % (Fig. 2b). More than half of the samples (56.5 %) were classified as "no profile" as no signals appeared in more than half of all loci. 37.0 % of the DNA profiles were "not interpretable", including two major profiles with five double-determined loci that narrowly missed the database inclusion criteria.

### 3.4. Factors impacting the quantity of background DNA

The distribution of the DNA quantities is plotted against the

categorical variables cleanliness, porosity, potential for background DNA, roofing and sky state (Fig. 3).

The DNA distribution for the weather data is shown in Fig. 4 plotted against the continuous variables temperature, humidity and rainfall. We fitted a zero-inflated general linear model to predict the DNA quantity with different variables. The model explains a significant amount of variance comparing to a null model ( $\chi^2 = 42.6$  on 10 degrees of freedom,  $p$ -value = 5.969E-06) (see [Supplements, Table 2](#)).

Only the variables temperature and porosity show a significant effect ( $p < 0.05$ ) (Fig. 5). More DNA was detected on non-porous stones than on porous ones (regression coefficient:  $-2.20E-01$ ,  $p$ -value: 6.89E-03). The temperature has the largest influence on the DNA quantity (regression coefficient:  $-4.58E-01$ ,  $p$ -value: 3.89E-06). The higher the temperature, the less amount of DNA was detected on the stones.

The linear model for the impact on the degradation index explains a statistically not significant and weak proportion of variance ( $R^2 = 0.10$ ,  $F(9, 46) = 0.56$ ,  $p = 0.819$ , adjusted  $R^2 = -0.08$ ).

## 4. Discussion

With a CODIS suitability for single/major component profiles of 20.6 % of the contact traces of stones analysed between January 2015 and March 2022, the database submission rate is slightly lower than the overall submission rate of the contact traces of the department in the same period with 22.3 %. Therefore, we can conclude that touch DNA sampling from stones is not remarkably less promising than from other surfaces.

The database submission rate is 3.2-times lower for the stones found in the city of Bern than for the casework samples. A similar value is obtained for the average amount of DNA detected on the city stones that is 3.6-times lower than the average DNA amount recovered from crime scene-located stones. The different submission rates are presumably because the probability for randomly collected stones of having been touched (or saliva or urine has been left behind) is lower than for the stones found at crime scenes. Most of the stones from previous cases are related to crimes like burglary or damage to property, where the stones may have been found inside a house after having been used as a tool to break a window for example. Those stones were definitely touched by

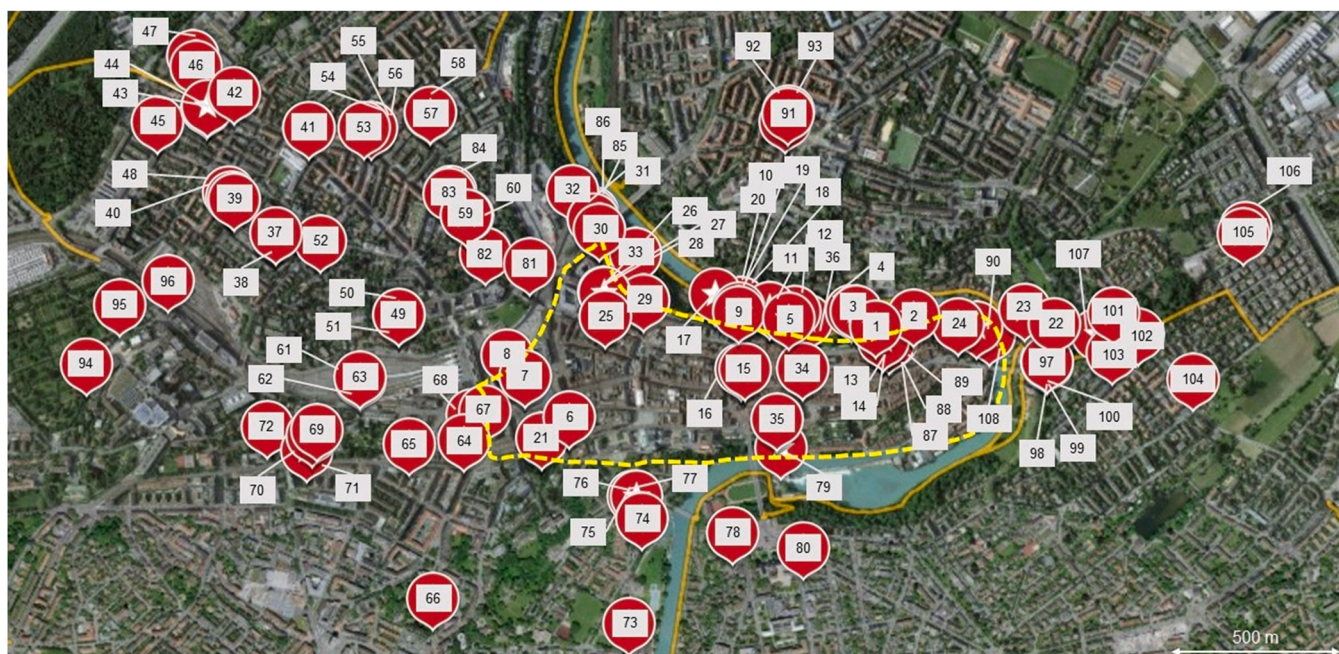
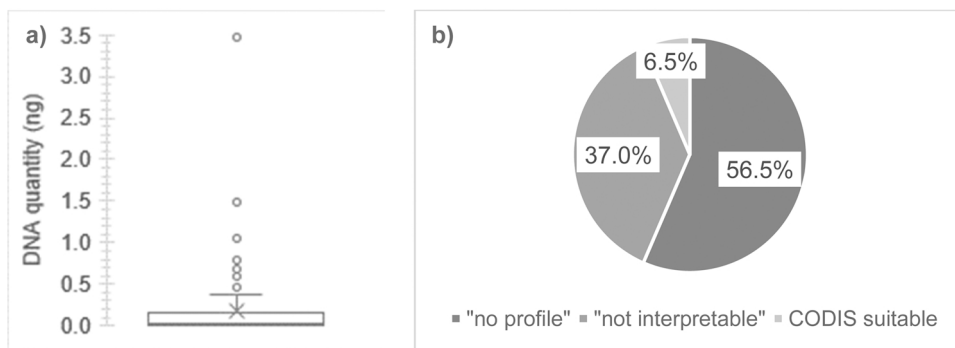
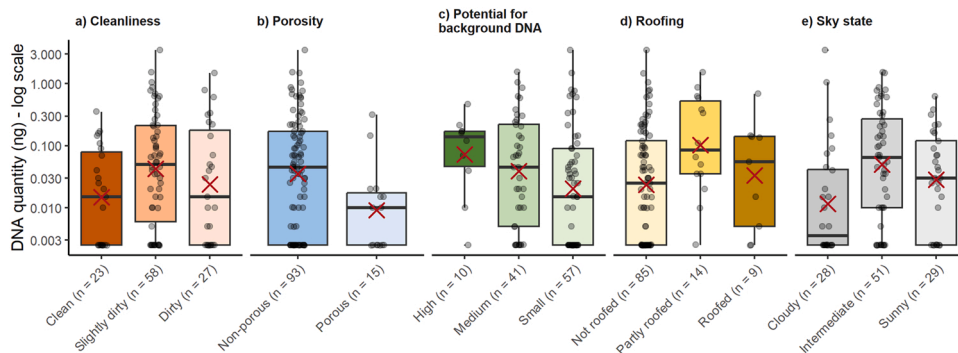


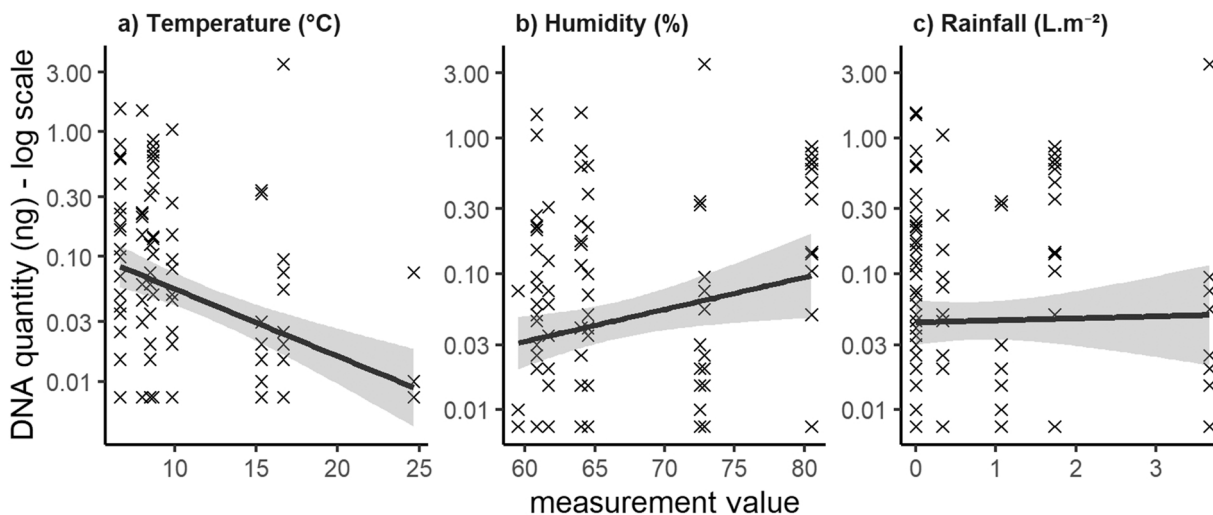
Fig. 1. Locations of the 108 sampled stones in the city of Bern. The yellow-bordered area in the middle shows the city centre with a lot of pedestrian traffic, buildings, shops, restaurants and the place where the most demonstrations take place. The river Aare is seen as bluish loop around the old city centre.



**Fig. 2.** a) Distribution of the DNA quantities of 108 stones from Bern with 0.181 ng on average. b) Classification of the STR typing results of the 108 stones from the city. "No profile" includes the profiles with no alleles detected in less than half of the loci. Seven profiles (6.5 %) fulfilled our CODIS entry criteria (see Methods and Materials).



**Fig. 3.** Measured DNA quantities, found on the 108 stones in the city of Bern. The entire dataset is included in each of the five categorical variables for a) cleanliness, b) porosity, c) potential for background DNA, d) roofing and e) sky state. For the logarithmic scaling, all data points with 0 ng were exchanged with 0.0025 ng.



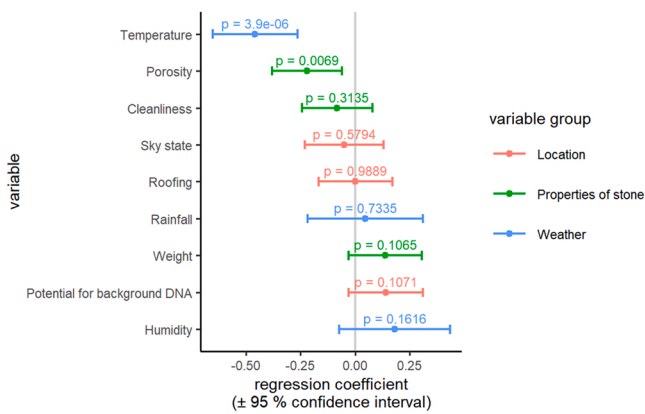
**Fig. 4.** Distribution of the DNA quantities, detected on the 108 stones in the city of Bern with twelve data points for each of the nine sampling days. Coincident data points (mostly zeros) are shown only once. The plots show the distribution for a) temperature (°C), b) humidity (%) and c) rainfall per day ( $L\ m^{-2}$ ) with a regression line and 95 % confidence bands. For the logarithmic scaling, all data points with 0 ng were exchanged with 0.0025 ng.

the perpetrator (assuming that the perpetrator was not wearing gloves).

We assessed the quality of the established DNA profiles from the point of view of their suitability for the Swiss DNA database, applying strict inclusion criteria. However, in addition to the 6.5 % of stones that carried a database suitable DNA profile, more than one third of the sampled stones carried a DNA profile that did not meet those criteria. At least some of these profiles might meet different criteria in other jurisdictions and might still be suitable for an exclusion of a suspect or an

evaluation of the potential profile contribution of a suspect by probabilistic genotyping. In addition, we did not assess the profiles for potential database suitable two person mixtures. Therefore, the percentage of stones from which useful DNA information could be retrieved might actually be significantly higher than 6.5 %.

According to our findings, DNA can be found on random stones in the city and it is possible to obtain a profile fulfilling the criteria for a database search, which thus can be used for interpretation reports in



**Fig. 5.** The influence of all measured variables on the DNA quantity. The regression coefficient with its  $\pm 95\%$  confidence interval of the single variables is depicted in the diagram. There is a significant difference ( $p < 0.05$ ) of the variables temperature and porosity. High temperatures and porous stones correlate with lower amounts of DNA.

court. This new information must be taken into account when interpreting the significance of such a DNA profile, especially in cases where the accused has or had legitimate access to the area of the crime scene. In the example of a decision of the High Court of the Canton Bern of 13 February 2018 (SK 2017 110), the defence claimed: "It could be assumed that quite a lot of DNA traces belonging to the suspect could be found in the immediate vicinity of his residence" [12]. Such a statement cannot be refuted per se, as demonstrated also by the results of this study. Simply applying the profiling success rate of 6.5 % from the stones sampled in the city to the retrospective casework data, we can hold that about one third of the CODIS suitable profiles, thus 99 profiles, could potentially originate from someone who was not involved in the respective crime. However, this extrapolation is much simplified and some limitations need to be mentioned, such as the fact that the stones from the real cases were found not only in crime scenes in the city, but also in the countryside, where less background DNA could be expected. In addition, if a person handles a stone with attached background DNA, this contact could lead to an abrasion of some DNA and an accumulation of his or her own DNA, thereby covering the DNA profile that might have been detectable before the handling.

Additional information can be received from the fact that the number of stones in the city is limited, so the possibility that the stones were brought to the scene specifically should be considered, mostly for cases where a couple of stones are involved, like in riots. It might be more likely that stones were brought than picked up on the spot, what might argue against a statement claiming that the suspect was present at the place previously, contaminating the respective stone days before the riot. On the other hand, given the possibility of background DNA on stones revealed, bringing stones to the city centre for the purpose of damage might place the DNA of people at the crime scene, who have never been to the respective city before.

The stone properties weight and cleanliness showed no significant impact on the DNA amount. However, the porosity of the stones, divided into porous or non-porous, induces a significant effect on the amount of DNA detected. Goray et al. [13] investigated the impact of the porosity on the transfer rate by comparing non-porous plastic and porous cotton. They stated that the transfer rate of skin cells is higher on porous objects. This contradicts the results obtained in this study, where on non-porous stones 5.5-times more DNA was detected. The material of stones is hard, compared to soft absorbent cotton, which could explain the different outcome. Increasing the sample number of 15 for porous stones could strengthen the informative value of our results. A conceivable possibility for the lower DNA quantities of the porous stones could be the sampling method by swabbing. For porous stones, the attached skin cells can be

stuck in the pores, leading to less efficient sampling with swabs. In addition, the swab is frayed more quickly on a rough surface, what might as well impair its sampling capacity. Further tests need to be conducted to test these assumptions. To improve the sampling method, other methods could be tested and compared, like the "Tape Lift Technique" with adhesive tapes [14]. Stoop et al. [15] showed that sampling contact traces on cotton results in better profiles with the SceneSafe Fast™ Minitape than with cotton swabs. The extent to which this might as well be the case for stones needs further investigation. The double swabbing technique could also lead to more DNA recovery [16]. Assuming that the police would swab the entire surface of a stone related to a crime, this might lead to a difference with our sampling method. We decided not to move the stones prior to sampling to prevent contamination or abrasion. To find out if there is a difference between sampling the whole surface and sampling only the visible surface, another study could be performed, examining the upper and lower surfaces separately. Another critical point is the classification of porosity, as this was determined subjectively in this study. An option to classify the surface roughness is the analysis by an atomic force microscope, described by Hughes et al. [17]. This option did not exist in our department, which is why we determined the porosity visually.

As already mentioned, most of the analysed variables showed no significant impact on the amount of DNA. The cleanliness of the stones does not seem to be an important variable, probably because the DNA analysis includes a purification step. The potential for background DNA was decided subjectively on the specific surrounding of the location, how many people there are and how likely it is for a person to come close to the stone. Although this factor could be important for the deposit and summation of DNA, the correlation results show no significant impact.

Environmental factors can have an influence on the DNA amount, persistence and the degradation. Factors like temperature, humidity, UV-light, microorganisms or pH-value can have an impact on DNA persistence [18,19]. Hydrolytic cleavage or oxidation based damage lead to DNA degradation [20]. We would expect moisture to lead to DNA degradation, but the tested influence of the humidity on the DNA quantity and degradation in this study showed no significant impact. Not only the humidity, but also the influence of rainfall and the roofing state of the stones were analysed but showed no significant influence. In fact, S96 was sampled after a rain shower and showed a profile with, according to our inclusion criteria, 11 database suitable loci. Consistent with our observation, Mcleish et al. [21] showed that interpretable profiles from contact traces can be obtained after ten days outside with heavy rain. Another study by Helmus et al. [22] showed the possibility to even detect a full profile on clothes with skin cells after two weeks in a pond.

The temperature of the three days prior to the sampling revealed to have the greatest impact on the DNA quantity. To find a balance between a snapshot and an average of some data points, we decided to analyse the impact of three days before the sampling day. The higher the temperature was the less DNA was detected. The same observation was made in a study by Zulkefli et al. [23] in which up to 100 % of the DNA was degraded when the temperature was increased from 5 °C to 35 °C in a  $10^6$  bacterial cell/ml containing medium, demonstrating the effect of promoted microbial activity by higher temperature. Therefore, increased microbial activity provides a good explanation for the significant influence of temperature on the DNA amount.

Assuming that DNA degradation increases together with temperature, we would expect also a temperature dependent increase of the degradation index, measured by qPCR. However, we detected no significant impact of the tested variables on the degradation index at all. The reason for this could be the fact that for 52 of the 108 samples, because of the low DNA quantities, no degradation index could be determined, limiting the validity of this observation considerably.

## 5. Conclusion

Human DNA can be found as background DNA on stones from public areas, regularly even in amounts that permit the establishment of CODIS suitable DNA-profiles. A significant influence on the amount of retrievable DNA is shown by the porosity of the stone and outside temperature. The success rate for DNA profiling from randomly sampled stones in the city corresponds to about one third of the success rate for crime-related stones from casework. The potential for background DNA on stones in an urban environment should be taken into account when evaluating such traces in forensic investigations and particularly in court.

## Ethics statement

The study does not fall under the scope of the Swiss Federal Act on Research Involving Human Beings. This study only involves anonymous trace material.

## Declaration of Competing Interest

The authors declare no conflict of interest.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.fsigen.2023.102880](https://doi.org/10.1016/j.fsigen.2023.102880).

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