MAPS FACULTY, DEPARTMENT OF NATURAL SCIENCES

DEPARTMENT OF SECURITY AND CRIME SCIENCES

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DNA DEGRADATION UNDER SHIFTING TEMPERATURE CONDITIONS

Casework implications of forensic DNA research to prevent miscarriages of justice

Alice Pistono[1,2], Bianca Szkuta[3,4], Georgina E. Meakin[5,6]

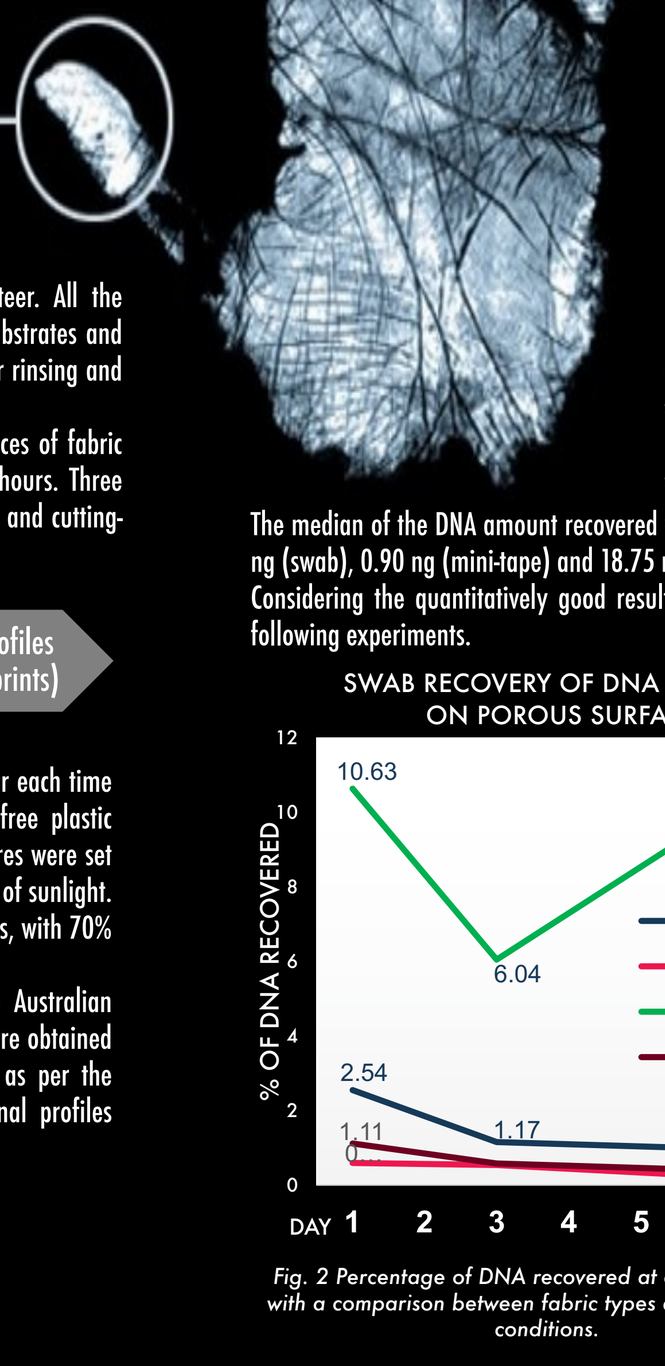
[1]UCL Department of Natural Sciences, faculty of Mathematical and Physical Sciences, Gower Street, London, WC1E 6BT
[2]UCL Division of Biosciences, Medical Sciences Building, Gower Street, London, WC1E 6BT, UK
[3]Office of the Chief Forensic Scientist, Victoria Police Forensic Services Department, 31 Forensic Drive, Macleod 3085, Australia
[4]School of Life and Environmental Sciences, Deakin University, Locked Bag 20000, Geelong 3220, Australia
[5]UCL Centre for the Forensic Sciences, 35 Tavistock Square, London, WC1H 9EZ, UK
[6]UCL Department of Security and Crime Science, 35 Tavistock Square, London, WC1H 9EZ, UK

INTRODUCTION

Forensic DNA research uses molecular biology techniques to aid the analysis of crime scene evidence. When collecting evidence from a scene, forensic geneticists use a variety of methods for DNA recovery. For porous surfaces (e.g., fabric), the commonest techniques are double swabbing, mini-taping and cutting-out of a substrate piece[1]. Over time, the amount of DNA that can be recovered decreases, due to the degradation of DNA molecules. The persistence of DNA is essential to ensure that any trace of genetic material is present on the crime scene. Temperature is one of the main variables affecting DNA persistence[2]. At 55° C, 13 days are sufficient to allow no DNA survival[3].

The project analyses the effects of changing temperature conditions on the persistence and degradation of DNA over time on two porous surfaces, cotton and polyester. A first experiment was conducted to assess which recovery method best suited the substrates involved. Subsequently, known amounts of DNA solution were deposited on the substrates. The fabric pieces were separately exposed to two oscillating temperature conditions (summer and winter) simulated by a climate chamber. Humidity and sunlight exposure were monitored at all times to isolate the temperature variables. DNA was recovered and extracted from each fabric piece at three time points (1, 3, 7 days after deposition). The experimental design mimics a non-sexual assault in which an offender attacks the victim by grabbing her clothes.

The project aims at improving the understanding of the relevance of DNA recovered on crime scenes exposed to changing temperature conditions. The comparison between cotton and polyester may suggest which fabric type should be prioritised for casework analysis. This piece of research may also be useful to prevent justice miscarriages based on the lack of experimental evidence as a benchmark for comparison.



METHODS

DNA solution was produced by extracting DNA from the buccal cells of a volunteer. All the experiments were conducted in triplicates to achieve statistical significance. All the substrates and materials were made DNA-free using a 10%-bleach solution followed by multiple water rinsing and UV-lighting.

For the first experiment, 10 microlitres of DNA solution were deposited onto two pieces of fabric (one cotton and one polyester) with a pipette tip. The surfaces were let dry for 2 hours. Three recovery methods were applied for DNA collection: wet and dry swabbing, mini-taping and cutting-out of fabric pieces. After recovery, all DNA samples were immediately extracted.

DNA deposition → Climate Chamber → DNA recovery → DNA profiles (fingerprints)

For the second experiment, the previous procedure was repeated, with 3 depositions for each time point (1, 3 and 7 days). The pieces of fabric were immediately placed into DNA-free plastic containers and transferred to a climate chamber. For the winter simulation, temperatures were set to shift from 0° C to 18° C in a 24-hour period, with 70% avg. humidity and 10 hours of sunlight. For the summer conditions, temperatures varied between 13° C and 35° C in 24 hours, with 70% avg. humidity and 16 hours of sunlight.

DNA recovery occurred using the double-swabbing technique (consistent with the Australian legislative habit). Samples were immediately extracted and quantified. DNA profiles were obtained using a 30-cycle PCR protocol and analysed with GeneMapper® 4.0 software, all as per the manufacturer's instructions. Analysis of Short-Tandem-Repeat allowed generating final profiles which in criminal cases correspond to the potential suspects' genetic "fingerprints".

RESULTS

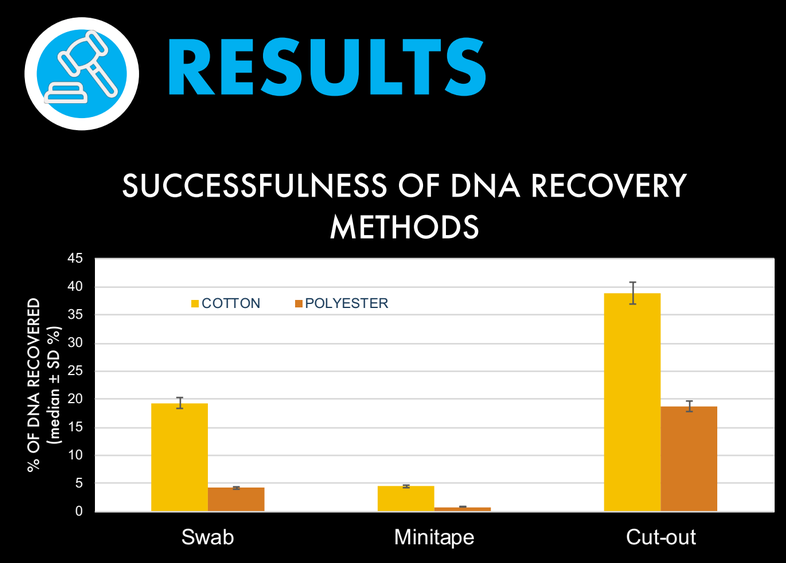


Fig. 1 Percentage of DNA recovered on each fabric type using three of the commonest methods available in forensic DNA analysis.

Fig. 1 shows the percentage of DNA solution recovered from the two substrates using different methods. The results are shown as a percentage of the amount recovered, with a median of the three replicates ± 5% standard deviation. Using an ANOVA test, it was found that the amount of DNA recovered on both substrates significantly varies with the method used ($p=6.31\times10^{-6}$ for cotton and $p=1.1\times10^{-6}$ for polyester).

The median of the DNA amount recovered was 19.34 ng (swab), 4.51 ng (mini-tape) and 38.86 ng (cut-out) for cotton and 4.22 ng (swab), 0.90 ng (mini-tape) and 18.75 ng (cut-out) for polyester.

Considering the quantitatively good results retrieved using the swabbing method, this was chosen for sampling DNA in the following experiments.

Fig. 2 Percentage of DNA recovered at each time point, with a comparison between fabric types and temperature conditions.

During the winter simulation, the cotton recovery retrieved median values for day 1, 3 and 7 equals to 5.53 ng, 3.14 ng and 5.74 ng, respectively. With polyester, the values were 0.58 ng, 0.30 ng and 0.18 ng. The decline in DNA recovery across the one-week period was found to be statistically significant for polyester ($p=5.52\times10^{-5}$). Cotton showed an initial decrease in DNA yield after 3 days, then an increase after 7 days, with an amount of DNA recovered greater than at t=1.

On substrates exposed to summer-like temperature conditions, the median amount of DNA recovered from cotton was 2.55 ng, 1.17 ng and 0.92 ng. For polyester, the values were 0.60 ng, 0.53 ng and 0.13 ng. On both substrates, DNA yield declined over time. The results proved statistically significant for the cotton recovery ($p=4.1\times10^{-3}$). For polyester, the p value was barely above the threshold of statistical significance, but this may be due to the minute amounts of DNA collected from this substrate (p=0.06).

By confirming the results obtained with the preliminary experiment, the recovery from cotton was significantly higher than from polyester, at all time points. As predicted, summer-like temperature conditions oscillating between 13° C and 35° C caused a visibly higher degradation of DNA, compared to winter conditions, more likely to preserve DNA molecules by maintaining 0° C for 6 hours each day.

CONCLUSION

- The DNA retrieved from a crime scene provides valuable information on the presence of suspects at the time of the offence.
- The degradation of DNA prevents its recovery from the crime scene. One variable negatively affecting DNA persistence is temperature.
- In summer (13° C < temp. < 35° C), DNA left on polyester is almost undetectable after 7 days. In winter (0° C < temp. < 18° C), DNA is well-preserved on cotton.
- DNA on polyester is hard to detect, thus cotton should be preferred for casework analysis.
- On porous surfaces, the best recovery method is the cutting-out of fabric pieces, but it is rarely used.
- When high temperatures are involved, well-preserved DNA retrieved from a crime scene may have been deposited after the crime was committed.

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