AMERICAN SOCIETY FOR MICROBIOLOGY | Applied and Environmental Microbiology®

MINIREVIEW

The Future of Environmental DNA in Forensic Science

Julia S. Allwood,[a] Noah Fierer,[b,c] Robert R. Dunn[d]

[a]Department of Molecular Biomedical Sciences, North Carolina State University, Raleigh, North Carolina, USA
[b]Department of Ecology and Evolutionary Biology, University of Colorado, Boulder, Colorado, USA
[c]Cooperative Institute for Research in Environmental Sciences, University of Colorado, Boulder, Colorado, USA
[d]Department of Applied Ecology, North Carolina State University, Raleigh, North Carolina, USA

ABSTRACT DNA sequencing technologies continue to improve, and there has been a corresponding expansion of DNA-based applications in the forensic sciences. DNA recovered from dust and environmental debris can be used to identify the organisms associated with these sample types, including bacteria, plants, fungi, and insects. Such results can then be leveraged to discern sample origin or geolocation and investigate individual identification. Here, we take a critical look at the current DNA-based technologies using microbiome and environmental sample sources that are focused on the generation of some investigative tools for use in forensic science. We discuss the pitfalls and contentions associated with the use of these techniques and highlight some of the future research required to expand the utility of these methods in the forensic sciences.

KEYWORDS environmental DNA, forensic science, eDNA, metabarcoding, DNA sequencing

A French criminologist in the early 20th century, Edmond Locard, was one of the first people to advocate the value of examining trace materials, such as dust and soil, to forensic science. Locard suggested that every contact between two objects left a trace of each object on the other, and that transfer between the two was inevitable. As he wrote, "For the microscopic debris that cover our clothes and bodies are the mute witnesses, sure and faithful, of all our movements and of all our encounters" (1). Locard was inspired by the writings of Sir Conan Doyle and the cases investigated by Sherlock Holmes (2), as well as by the work of Hans Gross, a famed Austrian criminologist who frequently included analyses of dust in his criminal investigations (1). With the value of trace material in mind, Locard categorized and catalogued the various elements of debris associated with different localities and occupations (1). Such elements of debris found by Locard included ova, larvae, feathers, hair, blood, microbes, epidermis, leather, tissue, fats, gelatin, muscle, bone, feces, insect matter, and textile materials (1), many of which were considered characteristic of particular regions, professions, or contexts. Locard used his catalogue of debris and its origins as a reference from which to draw comparisons from dust and microscopic debris gathered during criminal investigations (1).

For most of the history of forensic science, dust was analyzed by experts specialized on particular components of the soil biota, such as soil fauna, pollen, or fungal spores. Those organisms, or parts of organisms, were typically identified using a microscope or a magnifying glass. Morphological identification using microscopy was still the predominant form of analysis 60 years later when Bisbing (1989), a scientist from one of the leading entities in dust analysis at the time (McCrone Associates, Chicago, IL), wrote that dust accumulates everywhere and is representative of the "disintegration of the various components of our environment" (3). Today, novel DNA technologies allow the strains and species found in the biotic components of dust and soil to be identified

Citation Allwood JS, Fierer N, Dunn RR. 2020. The future of environmental DNA in forensic science. Appl Environ Microbiol 86:e01504-19. https://doi.org/10.1128/AEM.01504-19.

Editor Emma R. Master, University of Toronto

Address correspondence to Julia S. Allwood, allwoodjulia@gmail.com.

Accepted manuscript posted online 8 November 2019
Published 7 January 2020

based on their DNA sequence information, even if the strains and species that contributed this information are no longer alive and are represented by nothing more than traces of life (e.g., the leg of a rare tropical ant species or remnants of bacterial cells). Here, we critically review the potential of these environmental DNA analyses for forensic analyses, distinguishing between those approaches likely to be useful in the near future from those that, while exciting, seem less likely to lead to practical investigative applications in the next years or even decades.

The technology used to facilitate the testing of environmental material is often described as "metabarcoding" and uses high-throughput sequencing to simultaneously process hundreds of different samples. Metabarcoding generates sequence data from specific genes or gene regions, for example, cytochrome *c* oxidase subunit I for animals and 16S rRNA genes for bacteria. Such DNA sequencing approaches are now routinely used to address a variety of questions. Most prominently, metabarcoding can be used to infer which taxa (including plant, animal, and microbial taxa) are present in environmental samples. The identities of those taxa can be used to infer regional biodiversity, geolocation (estimating sample provenance or origin), and, potentially, even human identification. Metabarcoding often requires only minimal amounts of starting material and can be used with a wide range of sample types, including ice cores, soil, leaf litter, feces, settled dust, filtered air or water, human skin, objects, and clothing. This broadening scope of the use of DNA in general and sequencing in particular has flowed into forensic science, where it has the potential to offer solutions to the problems posed by Locard. To date, such forensic studies have focused primarily on the following two key objectives: (i) linking a person or object to a particular region or habitat (geolocation and/or sample provenance), and (ii) identifying the individuals who have touched particular objects based on their "microbial fingerprint." Here, we discuss some of the ongoing research relevant to addressing these key objectives, focusing on the use of environmental DNA for forensic science. We begin by considering attempts to establish sample provenance or geolocation, with a focus here on plant and fungal material. We then discuss work with the same objective of provenance but that was conducted using bacterial signatures in soil. Finally, we delve into the field of research focused on using microbial signatures for human identification and discuss future directions and requirements of some of these DNA sequence-based applications for forensic science. Our audience here is 2-fold. We aim to provide biologists who employ metabarcoding approaches with some context as to the needs and challenges of applied forensics. Simultaneously, we seek to provide forensic biologists with insights into the state of the art with regard to the use of metabarcoding-based approaches.

GEOLOCATION AND SAMPLE PROVENANCE

The use of plant and fungal evidence associated with the dust and soil at crime scenes has a long history, dominated by expert microscopy-based analyses via the comparison of taxon compositions of samples from crime scenes to reference material catalogues. Pollen and fungal spores are ideal for use in this regard due to their ubiquity, life histories, biogeographic distributions (with many taxa exhibiting a high degree of endemism), and environmental durability (4, 5). Examinations of both fungi and pollen material (included ingested material) have been used in criminal investigations for decades and are accepted as admissible evidence in judicial systems (5, 6). The use of this material as evidence, or as investigative leads, can be applied to large-scale geolocation efforts or in an attempt to identify a more local provenance (e.g., whether a sample was likely derived from a wetland as opposed to a forest). Pollen and fungal spore identification has been used as part of broader environmental profiling approaches. For example, pollen identification was used in the investigation into original and relocated grave sites unearthed in the former Yugoslavia following the cessation of the Homeland War (7). The pollen identified at the secondary (discovery) gravesites allowed materials associated with the bodies to be traced back to the original primary burial sites. Researchers have also attempted to create pollen calendars for investigative use, where the observation of particular pollen taxa on a corpse or object can be

correlated to a time of year given known flowering and pollen dispersal patterns. Such calendars could help ascertain the season of death based on the specific combinations of pollens found on a deceased individual (8, 9). In another case, pollen and fungal spores were used as evidence of a link between a suspect's shoes and the site of deposition of the victim's body (10). In this case, palynological material (spores, pollen, and other particulate material) obtained from the suspect's shoes was shown to match comparative material from the body and the site where the body was found. This discovery contradicted the suspect's statement that he had never walked near the site where the body was found (10). Another example involved aggravated burglary in which the composition of fungal spores at the crime scene was compared to that of spores collected at the suspect's stated alibi site. Fungi collected from the shoes and vehicle of the suspects matched the fungi of the scene of the crime but not the site described as part of the alibi (as summarized in reference 11).

However, visual identification of plant material and fungi in samples requires highly trained experts with access to identification guides and catalogues of reference materials (which may only be available for biologically well-studied regions), with morphological identifications often subjective and therefore at risk of being inconsistent across analysts, particularly for difficult-to-assign or damaged/incomplete specimens (5, 12).

One potential advantage of DNA sequencing approaches for determining the geographic origin and, more generally, the provenance of samples is that many taxa can be surveyed at once, and the composition of the assemblages of particular taxa can then be compared to that of particular geographic regions or habitats through formal statistical analyses. For example, an approach targeting fungal DNA extracted from dust was used by Grantham and colleagues (13) to predict the sample provenance, or geolocation, of nearly 1,000 dust samples from across the continental United States, with a median prediction error margin of 230 km. This study trained an algorithm to predict the most likely origin of samples based on the identity and composition of fungal taxa they contained (13).

The microbes and other taxa in soil, like those in dust, can also be used to identify the geographic origin of samples. Estimating the biogeographical provenance of soil has been attempted using various methods, including microbial DNA analyses (typically focused on bacteria) as well as analyses of soil chemistry, with various levels of success. DNA-based microbial analyses have been used previously to link collected material to a source location (many of these are reviewed in reference 14; refer to reference 15 for a recent example). Bacterial DNA analyses have also been used to link soil to source location for investigative use at crime scenes (16–18). Using a metabarcoding approach, initial studies observed that samples could, for the most part, be reliably differentiated from one another when collected from different locations or habitats (17, 18). Based on a relatively small number of samples, comparisons of bacterial communities could accurately classify over 95% of the samples to their original location (18). While temporal differences were noted, it was observed that the microbes detected via sequencing remained stable enough to still allow for classification (18). Given the incredibly slim likelihood that comparative samples would be collected from scene environments within the same immediate time frame as evidence samples, understanding temporal effects on bacterial signatures is of key importance. Badgley and colleagues (16) investigated the extent to which bacterial community signatures changed over time and collection context (e.g., soil collected on a shoe versus fabric, or stored in a warm versus cool environment) to mimic the processing and analysis of comparative material to evidence material. Changes in bacterial abundance and diversity were observed in all soil samples regardless of collection and storage, although it was noted that cooler storage conditions delayed this effect (16). However, regardless of the changes observed in diversity and abundance, these aged samples ($n = 364$) could nonetheless be correctly assigned to the original sample collection site in 95% of instances (16). In other words, the bacterial communities present in samples change after they are removed from their natural environment but perhaps not so much as to rule out their potential utility.

It is important to note that while some studies have demonstrated that bacterial analyses can be used to assign soil samples back to their original location, samples collected in close proximity to each other may often harbor highly varied microbial communities. The bacteria found in any given soil sample can be influenced by sampling depth, climate, vegetation, pH, and soil organic carbon concentrations (19). For example, soil samples collected from across Central Park (New York City, NY, USA) had an enormous breadth of prokaryotic community types (bacteria and archaea, inferred using 16S rRNA gene sequencing) and eukaryotic community types (18S rRNA), with an average of only approximately 19% of phylotypes ("species") shared from any two randomly selected samples (20). A comparison of the phylotypes observed from 52 randomly selected Central Park samples and 52 soil samples collected as part of a global collection effort (21, 22) illustrated that the majority of the more abundant phylotypes (over 94%) were shared across all soil samples. In contrast, only 4% of these phylotypes were found only in the global data set, and approximately 1% of phylotypes were observed only in the Central Park samples. Thus, based on these findings, it would be difficult to know if a given sample came from Central Park or some other location on the other side of the globe given the broad range of taxa found in soils from across the park. While several DNA targets were analyzed in this work, it would be interesting to determine if additional markers or variable analyses could improve the predictive capacity.

Identifying the geographic origin of samples based on sequence data from samples of dust, soil, or other material is limited by the need for suitable reference information for sample comparison (4, 12, 23). In other words, for the gene region being sequenced, a database of reference sequences of the same target is required, and, ideally, this reference database would cover all possible geographic regions under consideration. Despite these reference database requirements, such approaches are promising and generally compare favorably with more traditional approaches. Two studies that have compared pollen identification via microscopy versus DNA sequencing reported that the sequencing approach revealed a greater number of species than with microscopic analysis, with DNA sequencing being able to identify species undetected microscopically (12, 23). However, while the majority of taxa observed microscopically were also detected using sequencing, some key taxa were not identified using the sequencing approach (potentially due to the insufficient taxonomic resolution of the targeted marker gene region [23]).

Overall, the use of sequencing approaches to identify the composition of fungi, pollen, and potentially other taxa in dust shows promise for forensic use; however, additional research is required to identify the appropriate study design and reference sequence availability to avoid undue bias in application. In contrast to dust and other deposited samples, studies of soil have focused on the bacteria. The results of these studies suggest that bacteria might be useful in identifying the sources of soil samples, but variation in bacterial communities over small spatial scales (centimeters to meters) may present a persistent challenge. We suspect that the use of other organisms in soil samples in geolocation, such as fungi, may be more productive in the long term than is the use of bacteria. Barberán et al. (24), for example, found that across North America, fungi tend to have narrower geographic distributions than do bacteria (at least in dust) and vary more predictably with climate.

Undertaking comparative studies of traditional and sequence-based approaches to assess variation in recovery and detection would be advantageous to determine appropriate study design and provide some measure of bias and accuracy as a starting point for method development for use in criminal investigations. A key challenge moving forward will be understanding the real-world contexts in which these approaches are most useful, particularly the extent to which they are able to disentangle the effects of the accumulation of dust on people or objects at different times in different sites.

HUMAN IDENTIFICATION IN FORENSIC SCIENCE

Sequencing approaches for identifying individuals by targeting human DNA have been developed for application to forensic science and use in criminal investigations. In addition, the concept of the use of bacterial DNA associated with humans, predominantly skin microbial profiles, for human identification has emerged as the subject of ongoing research. This idea, however, rests on four core assertions, all of which must be satisfied if microbial profiling is to be used; however, none of them are yet sufficiently supported. First, it needs to be the case that each individual human has a unique microbial profile and that this profile does not change appreciably over time if static comparative databases are to be used. Second, that profile must be persistent and relatively consistent regardless of the contributing point of contact (which will be unknown in crime scene samples). Third, a comprehensive evaluated database of microbial profiles generated in a way that is regionally (or globally) useful is required. Fourth, any such profile must be the result of a method accepted within the community as robust, reproducible, and ultimately more useful than, or complementary to, existing technologies based on human DNA to be used in practice. Validation of all of these assumptions, while possible in theory, seems to be many years, perhaps decades, off in the future.

Work to date has focused on the simplest step, that of identifying whether or not the microbial profiles of individuals differ enough to be distinguishable. Early work demonstrated that the bacterial component of palm skin swabs from 51 healthy individuals showed an association with handedness and that the sex of the individual also influenced the skin microbiome (25). This work also demonstrated the presence of interpersonal differences in microbiome signature, with different individuals found to share only 13% of the bacterial taxa observed (25). This observable difference between the signatures across individuals led to the suggestion that microbial signatures could be applied as a microbial fingerprint to facilitate identification for forensic investigations (26). However, this lack of similarity is only 4% less than the similarity observed between the profiles compared between dominant and nondominant hands of the same individual, where only 17% of the profile was shared between hands of the same individual (25).

More recently, studies have considered the factors that influence the personal microbiome of individuals. We now know that the microbial community of an individual's skin is in part the product of the individual themselves and partly that of the individual's environment and lifestyle. The latter includes the interaction with personal objects (26, 27) and homes (28–30), influences that might be expected to change over time. In addition, it is known that individuals shed their microbes onto the objects they touch, such that it might be possible to identify who has touched an object. One study formally tested this possibility by trying to link three keyboards and nine computer mice to the people who had touched them, with the researchers finding that the signatures derived from each mouse were significantly more similar to the signature of the contributor than to those from a database consisting of 250 alternate individuals (26). While the results of this keyboard study indicated that it may be possible to identify an individual from within a small group (with known members who can be resampled or have been sampled in the past), this is not comparable to being able to carry out the same analysis in the context of a crime scene investigation to identify and compare a unique signature from across a larger population. Another study focused on mobile phones and found that when rarer taxa (representing $\leq$0.1% of an individual's sequences) were removed from the sample data, 82% and 96% of the microbial signature from the index finger and thumb, respectively (from the dominant hand), of participants were shared with the signatures obtained from their phones (27). Meadow and colleagues suggested that the assignment of phones to their owner or user could be achieved, as an individual had 5% more microbial data in common with their object than with objects owned by other individuals (27). However, this work also illustrated pronounced variance in a person's microbial signature between two skin sample sites

in close proximity, with an average of only 32% of bacterial taxa shared between neighboring fingers of an individual. Successfully linking signatures from owners of items (such as phones) has also been demonstrated in subsequent studies looking at the microbes found on shoes (31) and surfaces within the home (29). However, the authors of these same studies also note that the relationship between a personal object and its environment is dynamic and changes over time and the environment in question. For example, bidirectional transference is thought likely to occur between floor surfaces and shoes (31) and on surfaces within the home, with speculation that, over time, deposited traces would degrade or be replaced by additional transfers (29).

The majority of human identification work using microbial signatures has been undertaken using approaches that consider all bacteria within a sample. However, several new methods target particular bacterial taxa. One such approach, dubbed hidSkinPlex (32), consists of a panel of 286 markers targeting specific clades of bacteria. These targets were identified from a candidate gene trial (33) conducted from prospective targets mined from publicly available skin microbiome data from 12 individuals sampled across 17 sites at three different time points across two and a half years (34). The hidSkinPlex panel was evaluated on control bacterial strains and eight individuals sampled at three different body sites (foot, hand, and manubrium). This analysis was able to classify the resulting signatures to the correct contributor with 92%, 96%, and 100% accuracy for the three sample groups, respectively (32). As with other prediction methods, subsequent testing on additional samples not used for model training and from additional individuals will be important. A similar approach was developed for forensic applications, specifically targeting certain microbial taxa within feces, to provide additional information about the source/material type and to potentially discriminate individuals (35). Probes targeting a selection of microbial taxa were designed to yield a feces-specific signature to be applied to samples of unknown origin and to assess whether the signature was indeed fecal in origin and whether the overall microbial signature varied between samples (35). While further validation work is required, this method was applied to supplement human genotyping in two casework instances and proved to be a useful additional investigative tool for the identification of cell/material type (36).

Human identification using microbial profiles is generally proposed as an approach to complement traditional profiling using human DNA markers when human DNA could not be recovered or the data quality was low (32, 35). However, many variables still need to be assessed to appropriately evaluate the potential of such microbe-based identification methods, ideally using samples collected from large numbers of individuals. One such variable is whether the signature within trace material varies with time and environmental context. It has been documented that the human skin microbiome is highly diverse and varies over time (29, 30, 37). As a result, one might need not only a database of each human's microbiome but also a database that can be used to quantify how their microbiomes vary through time and to identify from which body site the sample was derived. For example, an investigation of household surfaces over different time points revealed that the accuracy of identifying a shared microbial signature from a household surface and that produced by a skin swab of the occupant(s) decreased substantially when the surface and occupant were sampled at different time points (29). Skin microbes, which are the most often used in forensic microbiome studies, also tend to be the most variable through time (at least compared to gut and oral microbes) (30). On average, only 15% of the phylotypes obtained from hand skin samples were observed again at other time points (1 to 6 weeks) in samples collected from the same individual, with microbial communities observed to be dynamic across time. It is important to note that it remains undetermined whether the 15% of phylotypes shared across samples in this context could allow for accurate individual identification. However, the microbiomes of certain individuals seem to vary more than those of others, and additionally, samples collected at closer time points, compared to those collected further apart, did not necessarily share more similar

communities (30). These temporal dynamics in human microbiomes are likely to make robust forensic analyses challenging.

With minimal shared similarity across the two hands of the same individual, and with high variability across skin samples of the same individual both generally and over time, extensive work needs to be undertaken to assess how a representative signature per individual would be established. It is unclear how such variation would be reliably accounted for in a forensic setting. Reproducible recovery and generation of microbial signatures still remain to be thoroughly evaluated, with many contributing variables to consider and map. Given that microbial skin profiles have been shown to vary per body site, demonstrate poor persistence/consistency on surfaces over time, are influenced by cohabiting individuals and environments, are confounded by multiple individuals handling/touching a surface or object, and have largely been evaluated using small numbers of individuals and in limited contexts, it seems implausible that microbial signatures are a viable human identification method on their own. Mixture studies illustrating the capacity to identify when a bacterial profile is the result of multiple individuals handling an object at different time points (for example), and then discerning the profiles within, will be of importance. The majority of this work was conducted using bacterial signatures. Human identification using other components of the microbiome, such as fungi, either applied alone or as part of a collective model with bacteria, has not been thoroughly evaluated. It appears that we are a long way from establishing some of the core precedents for an identification method, demonstrating a unique microbial signature that persists over time and place for standalone identification in a forensic context.

FUTURE DIRECTIONS

The ability to collect and extract DNA from trace material or microscopic debris and generate data using sequencing technology has wide-ranging applications for forensic use. From traces of biological material found in the dust of a home to the estimation of sample provenance from bacterial signatures in soil, this field of forensic research is expanding. As with all promising areas of forensic research, the focus eventually turns to questions of establishing standardized protocols and undertaking validation of methods to address the objective of acceptance for use in criminal investigations.

Many of the applications of environmental DNA and sequencing technology use small amounts of starting material and/or methods that can be difficult to standardize. The focus is then to target what can and should be standardized to ensure reproducibility, sensitivity, and, ultimately, accuracy. To this end, different groups and collaborative efforts have worked to evaluate appropriate extraction methods and DNA amplification approaches (for example, see references 38–40) to provide consistency and clarity where possible in the initial phases of such methods. Study and method design therefore are of key importance for validation work to ensure reproducibility and accuracy. It has been demonstrated that studies using high-throughput sequencing may be highly influenced by study design (41) and marker gene choice (23), which will have significant impact for forensic use. Therefore, studies designed to allow for appropriate forensic validation will be of key importance. There is also the need to establish methods that are robust to temporal and environmental factors or at least work toward establishing known limits of detection, expected error rates, and the applicability of each method under such conditions. Investigations into the temporal variation of the skin microbiome for human identification and soil microbiome for provenance are already under way; however, results have been inconsistent (discussed above). Many sequencing methods rely on reference databases which will need to be extensive and reliably compiled to facilitate appropriate use (4). The need for a validation strategy of these kinds of methods has been called for and stipulated as a requirement prior to accepted use as a forensic investigative method, with some ongoing efforts to facilitate this process (see references 42–44). Finally, as with the adoption of many new technologies, additional hurdles, including the degree of

acceptance of such methods within the community and legal system, are important considerations.

Combining the power and versatility of sequencing approaches with the ubiquitous nature of environmental DNA is a logical match for forensic biology. Scientists are often faced with trace or minute material collected for use in criminal investigations, and access to techniques to make the most of those samples is critical. The use of such material itself is not new or novel, but the use of DNA sequencing technologies may broaden, streamline, and standardize its use. The burgeoning field of sequencing outside forensics has exposed a variety of different applications that can be used to give more information beyond targeting human DNA. A growing field of research is focused on performing human identification without using human DNA at all, exploiting the microbial signature left behind by individuals in their environment and possessions. Research into moving the analysis of biological material beyond the conventional morphological identification using microscopy of plant and fungal matter, even from trace amounts in dust, has obvious advantages for specific forensic applications. However, many if not most of these methods still require careful consideration of temporal and environmental effects to determine their prospective feasibility when applied in an anonymous, context-free setting, while others still require conclusive proof of concept across large study populations. Ultimately, the use of these methods rests on the capacity to demonstrate that such methods are appropriately robust toward the impact of temporal and environmental factors while being accurate, reproducible, and reliable.

ACKNOWLEDGMENTS

We thank two anonymous reviewers for their constructive commentary and review of the manuscript, as well as Melissa Scheible for thoughtful review of an earlier version.

This work was partially supported by the U.S. Army Research Office and the Defense Forensic Science Center (DFSC) and was accomplished under cooperative agreement number W911NF-16-2-0195.

The views and conclusions contained in this document are those of the authors and should not be interpreted as representing the official policies, either expressed or implied, of the Army Research Office, DFSC, or the U.S. Government. The U.S. Government is authorized to reproduce and distribute reprints for Government purposes notwithstanding any copyright notation hereon.

REFERENCES

1. Locard E. 1930. The analysis of dust traces. Part I. Am J Police Sci 1:276–298. https://doi.org/10.2307/1147154.
2. Kaye BH. 2008. Tell-tale dust, p 82–85. *In* Kaye BH (ed), Science and the detective: selected reading in forensic science. John Wiley & Sons, New York, NY.
3. Bisbing RE. 1989. Clues in the dust. Am Lab 21:19–24.
4. Bell KL, Burgess KS, Okamoto KC, Aranda R, Brosi BJ. 2016. Review and future prospects for DNA barcoding methods in forensic palynology. Forensic Sci Int Genet 21:110–116. https://doi.org/10.1016/j.fsigen.2015.12.010.
5. Wiltshire P 2016. Mycology in palaeoecology and forensic science. Fungal Biol 120:1272–1290. https://doi.org/10.1016/j.funbio.2016.07.005.
6. Ladd C, Lee HC. 2004. The use of biological and botanical evidence in criminal investigations, p 91–109. *In* Coyle HM (ed), Forensic botany: principles and applications to criminal casework. CRC Press, Boca Raton, FL.
7. Brown AG. 2006. The use of forensic botany and geology in war crimes investigations in NE Bosnia. Forensic Sci Int 163:204–210. https://doi.org/10.1016/j.forsciint.2006.05.025.
8. Szibor R, Schubert C, Schöning R, Krause D, Wendt U. 1998. Pollen analysis reveals murder season. Nature 395:449–450. https://doi.org/10.1038/26646.
9. Montali E, Mercuri AM, Trevisan Grandi G, Accorsi CA. 2006. Towards a "crime pollen calendar"—pollen analysis on corpses throughout one year. Forensic Sci Int 163:211–223. https://doi.org/10.1016/j.forsciint.2005.11.020.
10. Wiltshire PEJ, Hawksworth DL, Webb JA, Edwards KJ. 2015. Two sources and two kinds of trace evidence: enhancing the links between clothing, footwear and crime scene. Forensic Sci Int 254:231–242. https://doi.org/10.1016/j.forsciint.2015.05.033.
11. Hawksworth DL, Wiltshire P 2015. Forensic mycology: current perspectives. Res Rep Forensic Med Sci 5:75. https://doi.org/10.2147/RRFMS.S83169.
12. Kraaijeveld K, de Weger LA, Ventayol García M, Buermans H, Frank J, Hiemstra PS, den Dunnen JT. 2015. Efficient and sensitive identification and quantification of airborne pollen using next-generation DNA sequencing. Mol Ecol Resour 15:8–16. https://doi.org/10.1111/1755-0998.12288.
13. Grantham NS, Reich BJ, Pacifici K, Laber EB, Menninger HL, Henley JB, Barberán A, Leff JW, Fierer N, Dunn RR. 2015. Fungi identify the geographic origin of dust samples. PLoS One 10:e0122605. https://doi.org/10.1371/journal.pone.0122605.
14. Young JM, Rawlence NJ, Weyrich LS, Cooper A. 2014. Limitations and recommendations for successful DNA extraction from forensic soil samples: a review. Sci Justice 54:238–244. https://doi.org/10.1016/j.scijus.2014.02.006.
15. Damaso N, Mendel J, Mendoza M, von Wettberg EJ, Narasimhan G, Mills D. 2018. Bioinformatics approach to assess the biogeographical patterns

of soil communities: the utility for soil provenance. J Forensic Sci 63: 1033–1042. https://doi.org/10.1111/1556-4029.13741.
16. Badgley AJ, Jesmok EM, Foran DR. 2018. Time radically alters *ex situ* evidentiary soil 16S bacterial profiles produced via next-generation sequencing. J Forensic Sci 63:1356–1365. https://doi.org/10.1111/1556-4029.13753.
17. Giampaoli S, Berti A, Di Maggio RM, Pilli E, Valentini A, Valeriani F, Gianfranceschi G, Barni F, Ripani L, Romano Spica V. 2014. The environmental biological signature: NGS profiling for forensic comparison of soils. Forensic Sci Int 240:41–47. https://doi.org/10.1016/j.forsciint.2014.02.028.
18. Jesmok EM, Hopkins JM, Foran DR. 2016. Next-generation sequencing of the bacterial 16S rRNA gene for forensic soil comparison: a feasibility study. J Forensic Sci 61:607–617. https://doi.org/10.1111/1556-4029.13049.
19. Fierer N. 2017. Embracing the unknown: disentangling the complexities of the soil microbiome. Nat Rev Microbiol 15:579–590. https://doi.org/10.1038/nrmicro.2017.87.
20. Ramirez KS, Leff JW, Barberán A, Bates ST, Betley J, Crowther TW, Kelly EF, Oldfield EE, Shaw EA, Steenbock C, Bradford MA, Wall DH, Fierer N. 2014. Biogeographic patterns in below-ground diversity in New York City's Central Park are similar to those observed globally. Proc Biol Sci 281: 20141988. https://doi.org/10.1098/rspb.2014.1988.
21. Dinsdale EA, Edwards RA, Hall D, Angly F, Breitbart M, Brulc JM, Furlan M, Desnues C, Haynes M, Li L, McDaniel L, Moran MA, Nelson KE, Nilsson C, Olson R, Paul J, Brito BR, Ruan Y, Swan BK, Stevens R, Valentine DL, Thurber RV, Wegley L, White BA, Rohwer F. 2008. Functional metagenomic profiling of nine biomes. Nature 452: 629–632. https://doi.org/10.1038/nature06810.
22. Lauber CL, Hamady M, Knight R, Fierer N. 2009. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. Appl Environ Microbiol 75:5111–5120. https://doi.org/10.1128/AEM.00335-09.
23. Richardson RT, Lin C-H, Sponsler DB, Quijia JO, Goodell K, Johnson RM. 2015. Application of ITS2 metabarcoding to determine the provenance of pollen collected by honey bees in an agroecosystem. Appl Plant Sci 3:1400066. https://doi.org/10.3732/apps.1400066.
24. Barberán A, Ladau J, Leff JW, Pollard KS, Menninger HL, Dunn RR, Fierer N. 2015. Continental-scale distributions of dust-associated bacteria and fungi. Proc Natl Acad Sci U S A 112:5756–5761. https://doi.org/10.1073/pnas.1420815112.
25. Fierer N, Hamady M, Lauber CL, Knight R. 2008. The influence of sex, handedness, and washing on the diversity of hand surface bacteria. Proc Natl Acad Sci U S A 105:17994–17999. https://doi.org/10.1073/pnas.0807920105.
26. Fierer N, Lauber CL, Zhou N, McDonald D, Costello EK, Knight R. 2010. Forensic identification using skin bacterial communities. Proc Natl Acad Sci U S A 107:6477–6481. https://doi.org/10.1073/pnas.1000162107.
27. Meadow JF, Altrichter AE, Green JL. 2014. Mobile phones carry the personal microbiome of their owners. PeerJ 2:e447. https://doi.org/10.7717/peerj.447.
28. Dunn RR, Fierer N, Henley JB, Leff JW, Menninger HL. 2013. Home life: factors structuring the bacterial diversity found within and between homes. PLoS One 8:e64133. https://doi.org/10.1371/journal.pone.0064133.
29. Wilkins D, Leung MHY, Lee P 2017. Microbiota fingerprints lose individually identifying features over time. Microbiome 5:1. https://doi.org/10.1186/s40168-016-0209-7.
30. Flores GE, Caporaso JG, Henley JB, Rideout JR, Domogala D, Chase J, Leff JW, Vázquez-Baeza Y, Gonzalez A, Knight R, Dunn RR, Fierer N. 2014. Temporal variability is a personalized feature of the human microbiome. Genome Biol 15:531. https://doi.org/10.1186/s13059-014-0531-y.
31. Lax S, Hampton-Marcell JT, Gibbons SM, Colares GB, Smith D, Eisen JA, Gilbert JA. 2015. Forensic analysis of the microbiome of phones and shoes. Microbiome 3:21. https://doi.org/10.1186/s40168-015-0082-9.
32. Schmedes SE, Woerner AE, Novroski NMM, Wendt FR, King JL, Stephens KM, Budowle B. 2018. Targeted sequencing of clade-specific markers from skin microbiomes for forensic human identification. Forensic Sci Int Genet 32:50–61. https://doi.org/10.1016/j.fsigen.2017.10.004.
33. Schmedes SE, Woerner AE, Budowle B. 2017. Forensic human identification using skin microbiomes. Appl Environ Microbiol 83:e01672-17. https://doi.org/10.1128/AEM.01672-17.
34. Oh J, Byrd AL, Park M, Kong HH, Segre JA. 2016. Temporal stability of the human skin microbiome. Cell 165:854–866. https://doi.org/10.1016/j.cell.2016.04.008.
35. Quaak FCA, de Graaf M-LM, Weterings R, Kuiper I. 2017. Microbial population analysis improves the evidential value of faecal traces in forensic investigations. Int J Legal Med 131:45–51. https://doi.org/10.1007/s00414-016-1390-8.
36. Quaak FCA, van de Wal Y, Maaskant-van Wijk PA, Kuiper I. 2018. Combining human STR and microbial population profiling: two case reports. Forensic Sci Int Genet 37:196–199. https://doi.org/10.1016/j.fsigen.2018.08.018.
37. Costello EK, Lauber CL, Hamady M, Fierer N, Gordon JI, Knight R. 2009. Bacterial community variation in human body habitats across space and time. Science 326:1694–1697. https://doi.org/10.1126/science.1177486.
38. Lear G, Dickie I, Banks J, Boyer S, Buckley H, Buckley T, Cruickshank R, Dopheide A, Handley K, Hermans S, Kamke J, Lee C, MacDiarmid R, Morales S, Orlovich D, Smissen R, Wood J, Holdaway R. 2018. Methods for the extraction, storage, amplification and sequencing of DNA from environmental samples. N Z J Ecol 42:10–50A. https://doi.org/10.20417/nzjecol.42.9.
39. Budowle B, Schutzer SE, Burans JP, Beecher DJ, Cebula TA, Chakraborty R, Cobb WT, Fletcher J, Hale ML, Harris RB, Heitkamp MA, Keller FP, Kuske C, LeClerc JE, Marrone BL, McKenna TS, Morse SA, Rodriguez LL, Valentine NB, Yadev J. 2006. Quality sample collection, handling, and preservation for an effective microbial forensics program. Appl Environ Microbiol 72:6431–6438. https://doi.org/10.1128/AEM.01165-06.
40. Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Huntley J, Fierer N, Owens SM, Betley J, Fraser L, Bauer M, Gormley N, Gilbert JA, Smith G, Knight R. 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J 6:1621–1624. https://doi.org/10.1038/ismej.2012.8.
41. Meisel JS, Hannigan GD, Tyldsley AS, SanMiguel AJ, Hodkinson BP, Zheng Q, Grice EA. 2016. Skin microbiome surveys are strongly influenced by experimental design. J Invest Dermatol 136:947–956. https://doi.org/10.1016/j.jid.2016.01.016.
42. Hardwick SA, Deveson IW, Mercer TR. 2017. Reference standards for next-generation sequencing. Nat Rev Genet 18:473–484. https://doi.org/10.1038/nrg.2017.44.
43. Breeze RG, Budowle B, Schutzer SE (ed). 2005. Microbial forensics, p 393–409. Academic Press, Burlington, MA. https://doi.org/10.1016/B978-012088483-4/50022-6.
44. Budowle B, Connell ND, Bielecka-Oder A, Colwell RR, Corbett CR, Fletcher J, Forsman M, Kadavy DR, Markotic A, Morse SA, Murch RS, Sajantila A, Schmedes SE, Ternus KL, Turner SD, Minot S. 2014. Validation of high throughput sequencing and microbial forensics applications. Investig Genet 5:9. https://doi.org/10.1186/2041-2223-5-9.