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Interview

BioTechniques

Unraveling crime scenes strand by strand: the forensic odyssey of Bruce Budowle

Bruce Budowle *,‡,[1,2]

[1]*Department of Forensic Medicine, University of Helsinki, Helsinki, Finland;* [2]*Radford University Forensic Science Institute, Radford University, Radford, VA, USA; *Author for correspondence: b.budowle@att.net;* ‡*Regents Professor, retired*

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ABSTRACT

Bruce Budowle speaks to Ashling Cannon, Journal Development Editor for *BioTechniques*, about advancements & challenges in forensic science.

Budowle completed his doctorate in genetics at Virginia Tech (VA, USA) formally known as Virginia Polytechnic Institute and State University. He then went on to complete a postdoctoral fellowship at the University of Alabama at Birmingham (AL, USA) to study genetic risk factors for acute lymphocytic leukemia, diabetes and melanoma. Budowle was early in his career and hadn't spent much time in forensics at this stage, but in 1982 an advert caught his eye for a job with the FBI to develop genetic marker systems to identify people who have left biological evidence at crime scenes. Budowle spent 26 years with the FBI and helped develop a plethora of genetic analysis methods. In 1985, it became a reality that DNA could be a signature for identifying people, and there were huge developments in DNA forensic analysis. In 2009, Budowle moved into academia and went to the University of North Texas Health Science Center (TX, USA), eventually becoming the Director of the Center for Human Identification, where he oversaw missing person and traditional crime cases, taught students and carried out fundamental and applied research. Budowle feels incredibly lucky to have had the resources, opportunities and academic infrastructure to learn and develop his knowledge. Budowle recently retired from academia and now spends his time building capacity for DNA forensics applications in Africa through the Department of Justice, with a well-established program known as the International Criminal Investigative Training Assistance Program (ICITAP) as well as with the non-government organization (NGO) DNAforAfrica.

KEYWORDS:

DNA analysis ● DNA sequencing ● forensic profiling ● genetic marker ● genetic profile ● next-generation sequencing ● PCR

What are the main techniques you use to solve cold cases & how does your approach differ when working with older DNA as opposed to newer DNA? Additionally, what are your thoughts on using DNA data banks to solve crimes?

While I personally dislike the term 'cold case' due to its negative connotation, it has been widely adopted by the media and is commonly used. However, it is important to shift our thinking and approach when dealing with these cases, if only to provide hope and resolution to the families, as the term 'cold' suggests that the case has been abandoned unresolved. Despite the popular terminology, there are technologies available that can aid in identifying individuals, including some that we started working on in the 1980s. In fact, one of my earliest forensics papers was published in *BioTechniques* [1,2].

Over time, the field of DNA analysis has witnessed significant technological advancements, leading to improved methods for identifying individuals. Although certain techniques are no longer in use due to continuous scientific progress, the underlying principles remain the same. The goal is to obtain a unique genetic profile that can be compared to DNA evidence. If the profiles match or are similar, the individual may be a contributor to the evidence. Conversely, if the profiles differ, the individual cannot be considered a contributor.

The foundations of DNA analysis were laid by Alec Jeffreys and the US company Lifecodes in the 1980s. This foundation, built in the 1980s and 1990s, brought forth more sensitive DNA analysis techniques, like PCR for DNA amplification. PCR essentially creates copies of specific targets in the DNA, enabling the generation of signatures or profiles for comparison. As technology evolved, the Human Genome Project facilitated the development of genetic genealogy and sequencing techniques. Next-generation sequencing (NGS) emerged, enabling high-throughput sequencing of multiple genetic marker systems and individuals simultaneously. This advancement revolutionized DNA analysis, allowing large portions of the genome to be scanned for genetic variants. Consequently, it became possible not only to determine potential contributors to the evidence but also to identify distant relatives who may serve as links to the individual in question.

This leads to the second part of the question. Regarding DNA databases such as 23andMe and ancestry.com, it is important to note that, to my knowledge, these platforms do not permit law enforcement to search their databases due to privacy and integrity concerns. However, independent hobbyists developed a database known as GEDmatch that allows individuals to upload their data from one platform to another. While the hobbyists used this feature to help individuals search for relatives, it was also publicly accessible

to US law enforcement, who began utilizing DNA profiles from crime scenes to uncover leads, resulting in successful resolutions of previously unsolved cases.

In the aftermath of the 2001 anthrax attacks in the USA, you pioneered the development of microbial forensics. Would you be able to discuss the process, the methods that were used & how those methods have developed since then?

During the challenging times following 9/11, when letters containing *Bacillus anthracis* (the bacteria causing anthrax) were being sent as a biological threat, a group of us collectively pioneered microbial forensics. We were woefully ill-prepared to deal with it on a forensic level, let alone on any other level. Although the available technologies were not fully developed, we leveraged the expertise of a colleague, Paul Keim, Regents Professor and Executive Director at the Pathogen and Microbiome Institute at Northern Arizona University (AZ, USA), who specialized in identifying bacteria by characterizing strains. This characterization revealed the rare Ames strain, which is particularly virulent and not commonly found in nature, making it of interest in biodefense. The strain was then sent to Ames National Laboratory (IA, USA). Its distinctive features pointed toward potential laboratory origins, narrowing the potential perpetrators.

The testing methods used were similar to those employed in human DNA analysis but adapted for bacterial DNA. The advent of NGS brought significant changes to the microbiological field, enabling faster and more comprehensive sample characterization. Today, this technology is routinely employed by the US FDA to trace outbreaks. We also explored non-DNA approaches, such as chemical characterization and isotopic analysis, to gather additional investigative leads.

While we initially lacked preparedness, the USA's abundance of intellectual resources, including governmental laboratories, universities and national research facilities, proved advantageous. I was responsible for bringing these entities together to develop practices, policies and technologies to support investigations, particularly the FBI's role, in the event of future crimes.

What do you believe to be the most significant landmark advancement in forensics in the last 40 years?

There have been several notable advancements. Prior to forensic science, pioneering work such as Southern blotting, restriction enzymes and the research conducted by Ray White's (Howard Hughes Medical Institute, University of Utah, UT, USA) laboratories set the foundation. However, within the field of forensics, the first major landmark was Alec Jeffreys' (University of Leicester, UK) work, which brought forensic DNA analysis to the forefront through his innovation and advocacy [3].

The invention of PCR was another crucial milestone. It allowed us to amplify tiny amounts of DNA, enabling analysis of samples that were previously thought to be impossible to yield genetic information. In the mid-90s, we developed a method to sequence mitochondrial DNA in hair shafts, which were previously believed to be devoid of DNA [4]. We now know that hair shafts contain highly degraded nuclear DNA.

Another significant advent was the introduction of automated capillary electrophoresis for DNA sequencing, pioneered by Leroy Hood and his colleagues at Caltech (CA, USA) [5], which today is the routine methodology used worldwide for forensic genetic analyses. Interpretation of genetic evidence found at crime scenes can be quite challenging and at times has been problematic. Therefore, the development of probabilistic genotyping, such as through the efforts of colleagues John Buckleton and Jo-Ann Bright at the Institute of Environmental Science and Research (ESR) in New Zealand, has significantly improved the interpretation of DNA evidence. This tool can democratize the interpretation process and aid in both routine and complex mixtures. Finally, we are seeing advances in technology with the advent of NGS; forensic genetics can advance to forensic genomics because of enhanced resolution and throughput. Clearly, NGS technology is fostering the development of additional innovative tools to identify previously unidentified persons of interest, combat human trafficking and address gender-based violence. There is still much to accomplish, but innovation has and continues to play a substantial role in enhancing capabilities to improve safety and security as well as bring resolution to victims, survivors, families and communities.

What is your perspective on technological advancements such as AI?

AI, or machine learning, is a significant innovation in the field. We were already utilizing it in microbial forensic research during my time at the university. For instance, it was used to characterize and identify microbes on the skin, enabling tracing back to individuals based on the microbial deposits left when they touch items that someday could be used to characterize evidence at a crime scene.

What considerations & challenges does one face when conducting sampling in laboratories versus the field?

There are distinct factors to consider when comparing laboratory and field settings. In the field, the process of identifying and securing a crime scene, along with collecting evidence to reconstruct events, can be chaotic and challenging. However, in the laboratory, the evidence is brought to you, and the focus shifts to analyzing the evidence itself, assuming it has been collected meaningfully. Nonetheless, there is always the possibility that the collected evidence may not be relevant or probative, which remains a consideration.

When validating methods, laboratory-generated samples are used to approximate real-world scenarios encountered in the field. However, it is challenging, if not impossible, to precisely replicate all the complexities of real-world events. Teaching individuals to understand

the methods and identify any anomalies is crucial. For instance, when concerned about potential microbial contamination leading to false-positive results, one may introduce specific microbes into human DNA samples and observe the outcomes. Yet, with countless microbial possibilities, it becomes difficult to mimic the exact conditions of the real world. Therefore, identifying signs that indicate discrepancies between the generated profiles and expected outcomes is essential. This is also observed in cases involving human remains and bones, where microbial components can influence the DNA profile.

The field presents additional difficulties as each case is unique, with varying circumstances. Factors such as the presence of DNA left by individuals prior to the crime, duration of exposure in the environment, presence of DNA-inhibiting chemicals, quality of remains and considerations regarding sensitivity and respect for families are all hurdles to overcome. For instance, while the petrous bone is one of the best for DNA typing due to its density, sampling it can damage the skull, creating challenges when families are awaiting the remains of their loved ones. Thus, a balanced approach that takes into account all aspects and implications is necessary, even in the field. Addressing these substantial challenges is a critical part of the process.

Aside from the petrous bone, what other sample types are known to yield high-quality DNA?

In addition to the femur, which is often discussed due to its compact bone nature, we have found great success with bones from the feet, that is the metatarsals. Teeth, particularly those without cavities, are also reliable DNA preservers.

It is also common practice to take multiple cuttings from a bone, if feasible, because DNA degradation is not uniform throughout. One cutting may yield no results, while an adjacent cutting could provide a complete profile.

What has been a significant moment in your career? Can you also tell us about what aspects of your career you have found most interesting & those you have found most challenging?

I consider myself fortunate to have been in the right place at the right time, joining the FBI and having the opportunity to establish a new discipline in forensics. I had the privilege of doing the same for microbial forensics and leading a center for human identification. Every case I have worked on has been important, as they involve victims, families and the need to protect society. It's difficult to single out one case, but the Scott Peterson case in California stands out due to its high profile and the legal challenges it presented to DNA evidence. Working on such cases required combining resources, scientific expertise and effective communication to navigate the adversarial legal system.

One challenge is presenting evidence in our legal system (that is the English-based systems). The scrutiny is quite high of the validity and reliability of the science used in criminal investigations and that rigor is a good thing. But scientists must be well trained, have fundamental understandings, use well-validated methods and, most importantly, be able to communicate the findings and validity to lay people. For some – probably most – people, this kind of environment is quite stressful. But if one has all the foundations to support the work, then it need not be so stressful. However, I remember fondly the early days of the challenges of the admissibility of DNA evidence in court proceedings. They were euphemistically called the DNA wars, but they were so important to laying the foundations of the field of forensic genetics as a science and as a reliable technology in the courtroom.

The highlights of my work have been mostly associated with the opportunity to work with so many brilliant people all over the world, some of whom I've mentioned. Their contributions have greatly impacted the field of forensics as well as my career. One influential person was Robert Allen (Medical University of South Carolina, SC, USA), a renowned expert in electrophoresis, who mentored me and instilled a passion for sharing knowledge.

The most challenging aspect has been effectively communicating the expertise and insights of myself and my colleagues, who were involved in forensic developments, to those who work in the field on a daily basis. Bridging the gap between developers and users of forensic techniques can be challenging due to differences in knowledge and access to resources. Additionally, our legal systems present challenges in incorporating the uncertainties and complexities of forensic evidence. It is crucial to find ways to integrate this understanding into training and practice, even when time constraints and limited resources pose obstacles.

In today's fast-spreading media landscape, who determines which cases are considered more 'important' than others? Is there a tendency to allocate more resources to cases with higher media coverage?

Yes, there is a tendency to prioritize cases based on media coverage. Ideally, every case would receive the same level of attention and resources, but the reality is that we need to establish systems, protocols and practices to efficiently analyze cases. Even with the available resources, there are often limitations due to the sheer number of cases.

The use of genetic genealogy, specifically the ForenSeq Kintelligence kit developed by Verogen (now owned by QIAGEN, CA, USA), shows great potential. Our analysis suggests that fully implementing this technology could potentially reduce the number of victims of crime, if acted upon, in the USA by an average of 50,000 per year, spanning rape, homicide, property crimes and more. While the investment required may be high, the cost savings from reducing victims by such a significant number would be substantial. Additionally, there are intangible benefits such as providing resolution to families and survivors and increasing the identification of perpetrators. Failing to prioritize these benefits means we contribute to the problem rather than being part of the solution.

What are the remaining gaps in the field & what are the advances you hope to see in the forensics field in the next 10 years?

There are significant gaps in technology transfer and training. Streamlining the validation process and facilitating the quick implementation of systems in laboratories are critical areas that need to be addressed. Newcomers in the field typically receive training from experienced professionals and tend to follow established practices without questioning them. However, as expertise grows, it becomes crucial to assess whether the approaches used are truly effective with current technological capabilities and to consider if they were applied appropriately in the past.

Another crucial issue is privacy concerns and ethical considerations. Informed consent has been a topic of discussion, not only in research involving human subjects but also in forensic practices. We need to acknowledge that legal permissibility doesn't necessarily equate to ethical soundness. For instance, validating studies often involve providing personal DNA samples, which may be subject to disclosure during legal processes. It is important to obtain informed consent and ensure individuals are aware of the benefits and, especially, the risks associated with their participation.

Moreover, historical abuses, such as the exploitation of vulnerable populations, have further highlighted the need for privacy protection. We must recognize that vulnerability can arise in various life circumstances and respect individual autonomy. The emergence of genetic genealogy has raised privacy questions, particularly regarding the genetic information of relatives. Balancing the benefits of forensic advances with individual rights and informed decision-making is crucial for the future.

Finding solutions to these challenges requires a balanced approach that considers diverse perspectives. By fostering informed discussions, collaboration and a focus on the greater good, we can navigate these issues and achieve meaningful progress.

Lastly, could you tell me about your experience with *BioTechniques*?

I was introduced to *BioTechniques* by Robert Allen, who mentored me and provided valuable guidance throughout my career. He said, "There's this new journal called *BioTechniques*," and I knew all these names like Eaton Publishing (the former publisher of *BioTechniques*), and James Ellingboe (the Scientific Editor at *BioTechniques* in the 1980s) and people like that, and now, as a member of the Editorial Board, I know Ebony and Ashling and the others as well!

The journal was a novel concept back then, embracing the idea of open access, although not in the online sense we know today. It was a magazine supported by advertising and offered free copies to readers, which was unique at the time. I'd argue that *BioTechniques* should receive the innovation award for open access because it started out as a magazine that was built on advertising to support it.

Having had a quick look online, I've published around a dozen articles in *BioTechniques*, the majority of them Methods, and some as recent as last year [1,2,6–17]. My first publication in the 1980s explored the improvement of microsample dialysis for enzyme polymorphism typing, alongside early DNA methods [1]. Additionally, we have published a mitochondrial DNA validation study, and recently a colleague of mine Magdalena Bus and I have contributed to the progress of forensic science by exploring new technologies for analyzing highly degraded samples [1,2].

From its inception, *BioTechniques* attracted passionate individuals who were motivated to contribute. It served as a platform where valuable articles could be submitted and promptly processed, ensuring widespread accessibility for everyone. It has been a remarkable success, exceeding expectations from the earlier days of publishing in 1983.

Financial & competing interests disclosure

Bruce Budowle is a member of the BioTechniques editorial board. Bruce Budowle also currently is a consultant with Verogen (owned by QIAGEN). The author has no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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