



Nonhuman forensic genetics

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ABSTRACT

Forensic genetics is the application of genetics to human and nonhuman material for the resolution (and prevention) of legal conflicts. Nonhuman DNA applications are increasing, from the ancillary role in criminalistics to the control of protected species and their products, microbial identification in bioterrorism or medical malpractice. We review this growing applications' scope and identify the major current difficulties, mainly resulting from the lack of standards and genetic databases as well as the poor or absent taxonomic definition of many major groups.

1. Introduction

When launching Forensic Science International: Genetics journal [1], forensic genetics was defined as: the application of genetics to human and nonhuman material (in the sense of a science with the purpose of studying inherited characteristics for the analysis of inter- and intra-specific variations in populations) for the resolution (and prevention) of legal conflicts. The study of nonhumans was already explicit, but the use was still limited and the field of applications quite narrow.

Significant progresses have happened: from the first 250 posters at this Congress portal (638), at least 33 (13.2%) deal with nonhuman DNA. Also at this Congress, quite a few commercial booths exhibit nonhuman DNA dedicated products. The use of nonhuman DNA increased, and the types of applications expanded, from the ancillary role in criminal investigations as 'silent witness', to the control of illegal traffic of protected species and their products, and microbial identification in bioterrorism or medical malpractice.

Excellent reviews have been published on the topic [2–4], entire books were dedicated [5], substantial sections of many textbooks address it [6,7] and databases initially designed for human identification include nonhumans (as Butlers' STRBase, <https://strbase.nist.gov/>).

Therefore, I will just make an overview of the growing scope of applications with some examples and identify the major current difficulties. Developments resulting from the new sequencing technologies will not be addressed, by lack of space and methodological instability still associated to these platforms [4,6]. Therefore, and due to other problems that will be made clear below, we will focus on complex, sexually reproducing organisms.

2. Fields of application

To encompass both investigative and probative roles of forensic genetics – it will be considered as (potentially) of forensic relevance any situation in which the same fact is interpreted or explained through (at least) two conflicting opinions. In this sense, it is hard to make a sensible classification of an expanding set of very diverse applications of nonhuman DNA. I list the following categories:

- Associative/transfer evidence (silent witness)
- Fraud/food and pharma security/GMOs
- Protected species and their products
- Fishing and hunting
- Identification (individual, breed, taxonomic) of organisms or their products; causing damage on property, body injury or death; theft; pathogens
- Detection of organisms' falsifications (identity, genealogies, lineages)
- Traceability...

I will center on the cross-topic of identification and nonhuman DNA as ancillary evidence.

2.1. Associative or transfer evidence (silent witness)

The first forensic uses of nonhuman DNA involved criminal cases where human DNA was (or thought to be) not available and therefore consisted in transfer evidence. The rationale is:

- The authors carry nonhuman biological material from the crime scene associable to it or to victim(s), or

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- The authors deposit at the crime scene nonhuman biological material associable to them

The first criminal case involved feline DNA (the ‘Snowball’ cat) and is a paradigmatic case of a happy conjunction of fortunate coincidences (white hairs in a dark background) in the forensic investigation [8] and, symptomatically, has not involved a forensic lab. Only later was this type of evidence admitted in US courts [9]. The use of nonhuman DNA is more and more important due to a CSI effect (criminals learned not to leave their biological traces) and to the abundance of pets and home plants. Dogs deserve a special note as they cumulatively can be the cause (but also the) victim of injuries or death to humans or property and can be confounded with the wild subspecies, the wolf.

2.2. Identification (individual, breed, taxonomic) of organisms

Taxonomic identification is relatively easy with (genetically well-studied) domestic species. This is the case of authentication and fraud detection of dairy products [10]. The same happens for breed affiliation [11] but not always [12]; it goes without saying that individual identification requires genetic databases or genealogical data [3]. Complexity arises when dealing with wildlife, despite efforts from the ecology field [13,14]. In fact, evolutionary theory is required to understand and mobilize genetic data, and even for sexually reproducing organisms, problems arise in the conciliation between classical (mainly morphological) taxonomy and genetic characterization [15]. This lack of translation between protected ‘species’ and genetically, legally defensible boundaries, drives most of difficulties in enforcement of wildlife protection laws, as can be seen in the UN World Wildlife Crime Report [16]. A significant progress was achieved through the International Barcode of Life project (iBOL) led by the Consortium for the Barcode of Life (CBOL) [17]. Nonetheless, the species coverage is still meagre and data quality quite variable across taxa. Species identification (a) is only possible if the unknown sample corresponds to a previously well characterized species (duly recorded in databases); (b) assumes that intraspecific differences are greater than interspecific (the barcoding gap, which is not the case of ‘young’ species with small divergence and with hybridization); and, due to (c) lack of standardization and technical problems with PCR based methods (false positives and negatives) it is particularly problematic in wildlife.

A serious source of worry of very special forensic interest – occurring also within domesticates – results from the fact that all methods of species identification based solely in uniparentally transmitted genetic markers do not correctly identify hybrids, but just one of their parental species. It is the case of mules – the offspring of a male donkey (*Equus asinus*) and a female horse (*E. caballus*) – which mtDNA based analyses would erroneously classify as *E. caballus* and, conversely, hinnies (offspring of male horse and female donkey) as *E. asinus*.

A final cautionary note: microbes are very poorly studied and their classification entails harsh analytical complexities, due to a fast evolutionary rate, high recombination and horizontal genetic transfer [18].

2.3. An illustrative example of lack of collaboration between forensic and conservation scientists

After anecdotal reports claiming that red foxes were deliberately released into the island of Tasmania, an eradication programme began in 2002. In order to detect the presence of foxes, a large pool of predator faeces was collected. In 2012, 56 of 7658 scats were assigned as ‘fox positive’ using a (supposedly) fox-specific PCR assay targeting mitochondrial cytochrome *b*. Unfortunately this assay proved to produce also clear amplifications for rabbit, cattle and pig as well as for the Tasmanian devil and a long-nosed potoroo. Therefore, this assay cannot be relied upon to detect red foxes because of the high frequency of false positives. Since wildlife management decisions are increasingly influenced by molecular data, these findings underline the need to adopt

best practice guidelines for forensic genetic investigations [19].

3. Current difficulties – a summary

The main problems of the field result from lack of: (a) cooperation between laboratories and scientists from different fields, (b) standards, (c) quality assurance/quality control programs, and (d) guidelines. To these systemic difficulties, we may add the taxonomic reluctance to introduce genetic criteria in taxa definition and delimitation as well as unstable nomenclature. Our poor knowledge on wildlife and microbes adds to this already long list of challenges. Concerning the specific field of GMOs (genetically modified organisms) detection, the new technologies of genome editing avoiding traceable insertion of exogenous sequences [20] such as CRISPR-Cas9 add extra problems to which solutions are still unseen and legislation not yet ready.

4. Examples of progresses to overcome the difficulties

A few institutions made some progresses establishing best practices, as the ENFSI manual [21], publishing recommendations [22,23], and organizing collaborative exercises, validation and proficiency tests [24–26]. Unfortunately, some efforts made by the ecology aimed institutions in delivering standards and guidelines do not profit from the experience gained in human forensics and display contradictory recommendations [27,28]. We hope scientists and professionals from both areas will be able to bridge these gaps, increasing and broadening collaborations between mutually dependent fields [13,14].

Declaration of Competing Interest

None.

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