WILDLIFE DNA ANALYSIS

Applications in Forensic Science

Adrian M. T. Linacre | Shanan S. Tobe

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Foreword

On 3 March 2013, in Bangkok, the 176 Parties to the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) will start their 16th meeting of the Conference of the Parties with a celebration of the fact that the Convention was signed 40 years ago.

One of the main difficulties in the global fight against illegal trade in wild animals and plants is – and always has been – the identification of the multitude of products derived from the 5000 animal and some 30 000 plant species covered by the Convention. The (sub-)species or products indicated on CITES permits and certificates may be completely different from the shipment they cover.

In quite a number of cases, commercial trade is prohibited when specimens are taken from the wild in certain countries but not in other range states. Also, trade in captive-bred animals may be allowed while specimens taken from the wild cannot be traded. In these cases it is necessary to be able to identify an individual as originating in a given wild or captive population, which requires a more complicated in depth analysis.

Fortunately, more robust methods than those relying on the morphological characteristics of particular species are being developed to assist wildlife trade controls. Methods such as those described in this book are crucial to identify specimens of species in international trade in contravention of CITES and of national conservation legislation.

Any identification method must be rapid, easy and most importantly accurate. It is vital that tests developed be so at the highest standards to ensure that any evidential analysis is beyond reproach. Error rates acceptable in other disciplines of science are not acceptable in criminal investigations and so comprehensive validation must be undertaken for any test used, something that is discussed in detail in this book.

Wildlife DNA Analysis: Applications in Forensic Science is a major contribution to more effective and reliable wildlife trade controls.

There is great interest in wildlife crime and interest in non-human DNA analysis is on the rise, which makes *Wildlife DNA Analysis: Applications in*

Forensic Science a much needed book. It fills a gap, bringing together the relevant aspects of wildlife analysis and introducing the rigor required in forensic science.

This book is a must for all those involved in wildlife trade controls.

Willem Wijnstekers
Former Secretary-General of CITES

Preface

Wildlife forensic science is a relatively new field in both research and its use in criminal investigations. It should be acknowledged from the outset that wildlife forensic science encompasses many areas of the analytical sciences and each plays a potentially crucial role. This book is focused on the use of DNA in wildlife forensic science while noting the importance of other areas of biological and chemical testing. It is also focused primarily on mammalian and avian DNA typing, again noting the importance and relevance of other species.

The book is aimed at those with an interest in DNA for human identification who are considering developing a wildlife forensic science capability. It is also aimed at those with little DNA knowledge but share an interest in wildlife criminal investigations. Only a scant knowledge of DNA is assumed in reading this book and every effort is made to explain terms and concepts.

While the application of DNA to wildlife forensic science investigations is relatively new, requests for assistance with such investigations are only likely to increase as the availability and appreciation of the technology also increases. It is paramount that all work conducted in the criminal justice system is to the highest standards expected and that there is no reduction in the quality of the work performed because the crime is against wildlife. It only takes one poor example of work presented in court to tarnish the rest of the members of the wildlife forensic science community. On a very positive note, there is such interest among forensic practitioners to become involved in wildlife forensic science investigations; this can only be beneficial.

The six chapters that comprise this book are designed to take the reader sequentially through the process of wildlife DNA typing, starting with introducing the rationale for the book and why we have become so passionate about our research and professional work in the investigation of alleged crimes involving wildlife. There are recurring questions in wildlife forensic science: *what species is this?* and *from what individual or population did this sample originate?* These questions can be addressed by DNA typing and the science behind the analysis to address these questions is the subject for the rest of the book. Chapters 2 and 3 describe aspects of the science behind the use of DNA in wildlife forensic science. These chapters are written with the assumption that the reader has little grounding in DNA and gives a historical context for the methods in current use. The use of DNA sequence databases to determine the probability that an unknown sample is from a particular species is illustrated in Chapter 4. This chapter is also designed to show step-by-step how different software programs are used in wildlife forensic investigations. Chapter 5 addresses the other main issues with wildlife DNA forensic science such an assigning a sample to an individual or a population. This type of sample assignment requires the use of software programs and equations, which will be explained to those unfamiliar with them. The final stage in forensic science is the evaluation and reporting of the results. This is described in Chapter 6 and draws on the experience of the authors. The need for high quality standards and best practice are common throughout the book.

The authors come from a forensic science background working in the area of human identification but developed an interest in wildlife investigation and scientific research. This profile led to requests and instructions to assist with criminal cases requiring the application of non-human DNA. We do not profess to have an in-depth knowledge of conservation genetics but wrote this book from a forensic science perspective. We very much hope that this book will lead to others gaining the same interest in this rapidly developing aspect of forensic science. We also very much hope that our work plays some part in the conservation of protected species and in the successful investigation of alleged crimes against wildlife.

About the Authors

Adrian M.T. Linacre is currently the South Australia Justice Chair in Forensic Science at Flinders University in Adelaide. His first degree was in Zoology at Edinburgh University before undertaking a PhD and three Research Fellowships at Sussex University, UK. Prior to moving to Australia he worked at the Centre for Forensic Science at the University of Strathclyde, UK for 16 years.

He has published over 90 papers in international journals with a wide range of these publications being related to wildlife research. He was chair of the International Society for Forensic Genetics Commission on the use of non-human DNA and has played a role in recommending standards and best practice in the forensic science.

He is co-author of the text *An Introduction to Forensic Genetics*, which is now in its second edition and is editor of the book *Forensic Science in Wildlife Investigations*. He has presented at a number of international conferences, is the President of the 25th Congress of the International Society of Forensic Genetics, and is Associate Editor of *Forensic Science International: Genetics*, handling non-human DNA publications. He is also on the editorial board of *Investigative Genetics*. His research is on trace DNA from both human and non-human sources.

Shanan S. Tobe is currently a Vice Chancellor's Post-Doctoral Fellow at Flinders University. He has studied forensic science for over 10 years specialising in forensic biology, genetics and in wildlife forensic science. He obtained his BSc Honours degree in Forensic Biology from Laurentian University in Canada followed by a MSc and PhD from the University of Strathclyde in Glasgow, UK.

Shanan is widely published in the area of wildlife forensic genetics and regularly presents at international scientific symposia. He holds membership with the American Academy of Forensic Sciences, the International Society for Forensic Genetics and holds professional membership with the Forensic Science Society.

His research focus is currently on the identification and individualisation of endangered species, and also on improving techniques for the recovery of DNA, both human and non-human, in relation to wildlife crime. Previously, Shanan developed a multiplex reaction capable of simultaneously identifying 18 different mammalian species common to the UK that were often associated with crime scenes.

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Our research could not be conducted without access to samples and in this regard we are grateful to Andrew Kitchener at the National Museum of Scotland and Rebecca Johnson at the Australian National Museum.

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1 Introduction

1.1 Importance of wildlife forensic science investigations

The scale of wildlife crime is difficult to judge accurately as so much may go undiscovered, unreported or unrecorded. The poaching of protected species by its very nature can occur in remote and isolated areas where there is little surveillance. As such, wildlife crime is more likely to be identified when samples are transported through border controls or other checkpoints.

Poaching of any kind can result in high financial rewards, a low chance of prosecution and penalties associated with convictions for wildlife crime are generally low. For these reasons there is an often quoted figure of something like:

'The illegal trade in wildlife is a US$20 billion a year industry, second only to trade in illegal drugs' (Zhang *et al.*, 2008; Alacs *et al.,* 2010).

The monetary figure will range between US$6 and 40 billion a year and is often attributed to Interpol, World Wide Fund for Nature (WWF) or another non-governmental organisation (NGO); however, Interpol have confirmed that they have never issued any statement to this effect (Christy, 2010). This figure, although believable (considering the cost of individual animal components), is difficult to estimate as monitoring the amount of illegal trade is itself the problem. It is at best an estimate as there are not the same international surveillance methods used in other areas of international criminal activity, such as drug enforcement or the trade in firearms, to investigate and prosecute offences involving wildlife. Organised crime has not been proven to be linked to wildlife crime, but there are indications that this could be the case (Sellar, 2009). Another influencing factor in wildlife crime is that there is a high financial return with little chance of capture, and even if captured, the

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penalties are light. Rarely does the maximum penalty for the alleged event meet the potential financial gains (Li *et al.*, 2000).

These financial gains can be highlighted by a number of examples of the illegal trade in wildlife or products derived from protected species. These examples include the illegal trade in the ultra-fine fabric to make a shawl called a shahtoosh, which requires between three to five killed Tibetan antelope (*Pantholops hodgsonii*) to make one shawl and a single shawl can retail at between US$8000 and $10 000. Single Australian parrot eggs could fetch as much as US$30 000 on the international market (Alacs and Georges, 2008). The cost of ivory on the black market remains high with ivory being marketed as from mammoth. Mammoth, since extinct, are exempt from international regulations and so can be imported, exported and sold legally. The cost of mammoth ivory is currently on average US$350 per kilogram (different 'grades' of ivory sell for different amounts and the highest grade can retail for over US$500 per kg), equivalent to US$350 000 per tonne, and worth US$21 million per year to the Russian economy (Martin and Martin, 2010). A clear issue is to be able to distinguish between mammoth and elephant ivory to ensure that ivory sold as mammoth is not actually from an elephant, but more importantly the need to ensure that the growing trade in what is described as 'mammoth' ivory does not lead to the increased poaching of elephants in Africa.

Traditional East Asian medicines (TEAM) still command a high price and an increasing market as populations in that part of the world increase. Other reasons include: human food consumption, such as bushmeat and shark fins; a symbol of wealth, such as dagger handles, ornaments and skins; as tourist curios which includes coral reef or wood carvings; and the live pet trade that includes snakes, geckos, parrots, and even primate species. As the deterrent is low with low levels of detection and minimal fines or prison sentences if caught, there is reason to believe that organised crime groupings are involved with illegal trade in wildlife due to the large financial rewards. For many species poached illegally, as they become more rare in the wild so they attract a higher value on the black market, and hence the trade is more lucrative to those unconcerned with their conservation (Courchamp *et al.*, 2006). This is coupled with the problem that many highly prized (by some) species naturally occur in countries where the average wage is low and hence the financial attraction in poaching a wild animal is great. A distinction should be drawn between low level hunting by a local community who consider such activities as an ancestral right and harvesting on a commercial scale that has a detrimental effect on species numbers or activity driven by financial gain only.

The effect of trade in wildlife on particular species has been great, although in some cases the rapid decline in numbers is also associated with habitat loss. According to the recent census by the WWF only 3200 tigers (*Panthera tigris* spp.) exist in the wild. This is a reduction of over 90% from the last century, leading to more tigers existing in captivity in Texas alone than in the wild. The population of black rhino (*Diceros bicornis*) decreased by 96% between 1970 and 1992 (International Rhino Foundation); in 1970, it was estimated that there were approximately 65 000 black rhinos in Africa – but by 1993 there were only 2300 surviving in the wild. Intensive anti-poaching efforts have had encouraging results since 1996. Numbers had been recovering in some areas but not in countries where there is limited or no protection from poaching. The increase in the desire and cost for rhino horn has recently resulted in a significant increase in the killing of rhino. In South Africa the number of rhino shot for their horn was 13 in 2007, but this rose to 83 the next year, 122 in 2009, 333 in 2010, 448 in 2011 and is over 250 for the first half of 2012 (information from Dr Cindy Harper of the University of Pretoria). The estimated black market cost for genuine rhino horn is between US $20 000 and $90 000 per kilogram. Survival of those rhino that remain is due in no small part to the dedication of wildlife officers in the field (see The Thin Green Line web site www.thingreenline.info). In the case of the Western Black Rhino it was officially declared extinct on 10 November 2011 by the International Union for Conservation of Nature (IUCN). The organisation stated further that two other subspecies of rhinos were close to meeting extinction. Central Africa's Northern White Rhino is possibly extinct in the wild and the Javan rhino is now thought to be extinct in Vietnam, after poachers killed the last surviving one there in 2011 for its horn. Tiger and rhino highlight the problem as they are high-profile species, but the situation is reflected with similar declines in many avian, reptilian and amphibian species. Some examples of products derived from wildlife contrary to CITES regulations are displayed in Figure 1.1.

1.2 Role of forensic science in wildlife crimes

Given the estimated size of the trade in wildlife, and the threat to species, it would be assumed that there is investment in forensic science to aid in combating these illegal activities. The types of forensic science methods pertinent to the enforcement of wildlife legislation include: veterinary pathology, where persons skilled in this discipline perform a similar role as their human counterparts and determine cause and time of death; crime scene examination, to record and collect evidence such as latent fingerprints and DNA, both of the animal and potential human DNA from the perpetrator (Tobe *et al*., 2011); morphology/microscopy, as simple comparison of hairs, furs and feather is often the first step in determining what species is present; ballistics, in the comparison of bullets recovered from carcasses to cartridge cases found at a poaching scene and a particular firearm if seized subsequently; document examination, to determine authenticity of documents relating to the trade in species; chemical profiling, to determine possible geographical origin based on isotope ratios; and DNA analysis to determine species and potentially link to a particular individual in a similar manner as their human

Figure 1.1 Examples of the types of wildlife products seized at a busy airport (Glasgow International Airport) that are contrary to CITES and National Laws. These products are often submitted to forensic laboratories for analysis to determine if they are in violation of any laws. This includes traditional medicines and tonics (top), products made from ivory and horn (middle) and stuffed animals, clothing and wallets (bottom). © S. S. Tobe, with permission. Items shown courtesy of the UK Border Agency.

counterpart. It is this last part that will be the focus of this book although it is important to realise that forensic science has many techniques that can be complementary. Forensic science has a range of tools and it is essential that the appropriate tool is used to address the allegation.

1.3 Legislation covering wildlife crime

Forensic science can only be employed if there is reason to believe that a piece of legislation has been breached and that there is a need for an investigation to determine whether a crime has occurred, and if so who committed the crime. Legislation relevant to wildlife crime falls under two broad areas: international and national.

Table 1.1 Number of species in the three CITES appendices. Based on data from CITES Secretariat.

	Appendix 1	Appendix 2	Appendix 3
Mammal	297	492	44
Bird	156	1275	24
Reptile	76	582	56
Amphibian	17	113	1
Fish	15	81	–
Invertebrate	64	2142	22
Total fauna	625	4685	147
Total flora	301	29 105	119
Total species	926	33 790	266

Note this information is up to date as of 2011.

The international organisation that oversees the trade in protected species is the Convention on the International Trade in Endangered Species of Flora and Fauna (CITES). Founded in 1973 it currently has 175 countries (known as Parties) as signatures to the Convention. The role of CITES is to monitor trade in species and recommend a ban of all trade in particular species when necessary. There are three appendices that underpin the role of CITES. Appendix I lists species that are threatened with extinction if trade is not prohibited. Trade is permitted only in exceptional circumstances such as the movement of samples/organisms for research or conservation purposes. Species on Appendix II are those that are not necessarily in danger of extinction but could become so if trade were not strictly regulated. Appendix II also contains some species that are not in themselves threatened but have similar morphology to a species that is endangered and hence allows better enforcement of trade for the endangered species. Those species on Appendix III are species which individual Parties to the Convention choose to make subject to regulations and for which the cooperation of other Parties is requested in controlling trade. Comprehensive information on the role of CITES with links to Appendices I, II and III can be found at www.cites.org. The numbers of some of the species listed currently under the three appendices are provided in Table 1.1. An example of the legislation and role of CITES in the trade of timber, and particularly mahogany products, is shown in Box 1.1.

Nationally, countries are required to enact laws in order to implement and enforce CITES, and additionally many countries have enacted laws to protect other wildlife within their borders. In Australia, the international movement of wildlife and wildlife products is regulated under Part 13A of the Environment Protection and Biodiversity Conservation Act 1999 for all wildlife, including cetaceans. The Act regulates: the export of Australian native species other than those identified as exempt; the export and import of species included in the Appendices of CITES; and the import of live plants

Box 1.1 The trade in timber products providing details of the legislation and role of CITES to regulate trade in legally sourced timber

Recent estimate are that approximately 50% of timber exports from the Amazon Basin, Central Africa, South-East Asia and the Russian Federation originate from timber that has been logged illegally (Li *et al*., 2008; Goncalves *et al*., 2012). The scale of illegal logging is believed to be one of the chief causes of worldwide deforestation. Additionally the trade in illegal timber and wood products creates market disadvantages for products from legal and sustainable forestry. The WWF estimates the global costs of illegal timber at approximately €15 billion per year (wwf.panda.org), although this figure is an estimate and cannot be verified easily.

European Union (EU) timber regulations (No 995/2010) were enacted in December 2010 and will come into force in March 2013 to make it illegal to place illegally harvested timber and timber products on the European market. The new rules target the trade of illegally sourced timber and place responsibility on traders and importers to perform due diligence by seeking guarantees that the timber products they sell have been harvested in a sustainable way and according to the laws of the country of origin. The EU has negotiated a Voluntary Partnership Agreements (VPA) with individual timber-producing countries. VPA countries agree to export to the EU only verified legal timber with a Forest Law Enforcement, Governance and Trade (FLEGT) licence. It should be noted that the new EU rules are modeled on similar legislation adopted in the United States in 2008, as an amendment of the Lacey Act. This amended Act prohibits in the United States all trade in plants and plant products, including timber and timber products, that are sourced illegally from any US State or foreign country. Further, the amended Act requires importers to declare the country of harvest and the species name of all plants contained in their products. This maximizes the opportunity of tracking legally traded timber to the place of origin.

The trade in mahogany species is regulated by CITES with members of the genus *Swietenia* on Appendix II. Species on this Appendix allows commercial trade from some plantations only if subject to appropriate controls. Examples of these include the prohibition of all imports into the EU of *Swietenia macrophylla* from Bolivia (enacted in August 2010). The problem of identification of mahogany species and products from these trees has led to CITES regulating the trade in look-alike species.

and animals that (if they became established in Australia) could adversely affect native species or their habitats (hence a threat to the biodiversity).

In the United Kingdom, the Wildlife and Countryside Act 1981 and recent amendments (1985, 1991 and 2010) are the main pieces of legislation for the protection of wildlife. Additionally, separate Acts have been established for particular species (e.g. seals and badgers (1970, 1992)) or activities, such as hunting or scientific research. Most countries have their own laws pertaining to the enforcement of CITES and the protection of wildlife within their borders. Examples include the Indian Wildlife (Protection) Act (1972) amended in 1993 and 2002; the United States Endangered Species Act 1973; in Canada there is the Canada Wildlife Act 1985, Wild Animal and Plant Protection and Regulation of International and Interprovincial Trade Act 1992 and Wild Animal and Plant Trade Regulations 2009; Ireland has the Wildlife Act 1976; and Thailand has the Royal Decree for Wildlife Preservation and Protection B.E. 2535 (1992) amended 2003.

In addition to national and international legislation the European Union also has an Environment Directorate General (http://ec.europa.eu/environment/cites/home_en.htm) that regulates trade into and out of the 27 member states. There are several regulations that have been enacted for different aspects of wildlife protection and conservation. These include:

- Council Regulation (EC) No 338/97 of 9 December 1996 on the protection of species of wild fauna and flora by regulating trade therein, in (EC) No 338/97 1997: European Union.
- Council Regulation (EC) No 812/2004 of 26.4.2004 laying down measures concerning incidental catches of cetaceans in fisheries and amending Regulation (EC) No 88/98, in 812/2004. 2004: European Union. p. 1–20.
- Commission Regulation (EC) No 1579/2001 of 1 August 2001 amending Council Regulation (EC) No 338/97 on the protection of species of wild fauna and flora by regulating trade therein, in (EC) No 1579/2001. 2001: European Union.
- Council Directive 79/409/EEC of 2 April 1979 on the conservation of wild birds, in 79/409/EEC. 1979: European Union.
- Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora, in 92/43/EEC. 1992: European Union.

In order to aid in the enforcement of wildlife crime the CITES Secretariat has a Memorandum of Understanding with the US National Fish and Wildlife Forensics Laboratory (www.labs.fws.gov) in Oregon. This laboratory is devoted entirely to the investigation of wildlife crime and provides a free service to any Party of CITES. There are few such dedicated wildlife forensic science laboratories in existence; normally much of the investigation, if performed, is sent to the operational police laboratories or units within universities. The UK Partnership Against Wildlife Crime (PAW) provides financial assistance with wildlife crime cases. More recently the Society for Wildlife Forensic Science (www.wildlifeforensicscience.org) (see Section 1.7 for more details on relevant societies) has been established to bring together those with a common interest in this particular area of forensic science. A common phenomenon with specialist wildlife laboratories is that they can be underutilised as they can only examine the samples that are collected and submitted. It is the examination of crime scenes in remote areas that is more of a problem. This is particularly evident in underdeveloped countries where crimes against people and property may not be examined due to lack of resources, far less alleged wildlife crimes.

The wording of any relevant legislation is essential as this forms the question to which forensic tests are addressed. The purpose of any further testing is to determine whether there is scientific support for the allegation or whether the scientific data supports a credible alternative scenario. The allegation must relate back to a particular piece of legislation.

1.4 Role of non-human DNA in forensic science

The use of DNA in a forensic investigation from species other than human is widespread and falls under broad themes.

The first theme is where non-human DNA is associative evidence in crimes against people or property. Pets are common in many households in North America and Western Europe with 77.5 million domestic dogs living in the United States alone (American Pet Products Association (APPA), 2010). Since most breeds of domesticated dogs and cats shed hairs readily, it would be difficult to enter such a home and not have pet hairs transferred to clothing. These hairs can then be deposited elsewhere and hence pet hairs are frequently found associated with crime scenes. The first use of animal hairs in a forensic investigation was that of Snowball the cat, as a result of the pioneering work of Menotti-Raymond and colleagues (Menotti-Raymond *et al.*, 1997). In this instance, genetic testing of a known cat called Snowball was linked to cat hairs on a jacket in relation to a homicide investigation in Canada. Since then canine DNA typing has been used to link dog as associative evidence in a number of forensic investigations (Wetton *et al.*, 2003, Berger *et al.*, 2008). These types of analyses now open the chance to use DNA from domestic species in mainstream forensic DNA typing (Halverson and Basten, 2005). Other associative evidence includes pollen, leaf or pine needles. Botanical evidence is an underutilised resource, yet is commonly found on evidential items and can be a rich source of DNA (Azad and Bhadauria, 2008, Craft *et al.*, 2007, Tsai *et al.*, 2006b).

The second theme is in regard to crimes against animals, whether by humans or by another animal at the direction of humans. This can include for example thefts, cruelty, hunting, fighting, worrying and poaching, whether for meat, pleasure or trade. In the United Kingdom fox hunting, hare coursing and badger baiting, which all use dogs to either hunt or fight with another animal, have all been deemed illegal; however these activities still take place. Being able to identify the DNA of dog on a fox carcass, for example, could indicate that fox hunting took place. If that dog DNA could be profiled and matched to an individual dog then police could proceed with filing charges. Similarly, poaching is a problem in many countries, and not just with protected or endangered species, and recent research has demonstrated that human DNA can be recovered from animal remains, which when coupled with identifying traces of the poached animal on a suspect can prove strong evidence (Tobe *et al*., 2011, Tobe and Linacre, 2007).

The third and final theme is in the movement and trafficking of wildlife. This relates not only to protected and controlled species, but also uncontrolled and unprotected species. Countries such as Australia, invest a large amount of money and resources into keeping unwanted and potentially invasive species out of their borders. Investigations into the possession of protected or controlled species may now use DNA as part of the forensic tool kit. An example includes illegally logged tress (Eurlings *et al*., 2010, Finkeldey *et al*., 2010, Stambuk *et al*., 2007). Agarwood is an example where all members of the two genera that produce agarwood (*Aquilaria* and *Gyrinops*) are listed on CITES Appendix II. As it is a commercially grown crop, DNA testing is used in an attempt to distinguish cultivated agarwood from illegally logged samples. DNA testing has been used in cases of alleged possession of cannabis (Howard *et al*., 2008, 2009; Gigliano, 1999; Hsieh *et al*., 2003b; Mendoza *et al*., 2009; Tsai *et al*., 2006a) including the illegal supply of this controlled substance. Other examples include the isolation of DNA from shells of protected species (Hsieh *et al*., 2006; Lo *et al*., 2006), scales of protected mammals (Hsieh *et al*., 2011), sculptures of ivory (Lee *et al*., 2009) and horn (Hsieh *et al*., 2003a), clothing such as shawls (Lee *et al*., 2006), food products (Moore *et al*., 2003; Chapman *et al*., 2003), and medicines (Tobe and Linacre, 2011).

Due to the wide variety of uses of non-human DNA in forensic science investigations, this book will focus on the specific use of DNA in wildlife crime and in particular on mammalian and avian species, although reference will be made to invertebrate and botanical uses where appropriate.

1.5 Development of wildlife DNA testing

Forensic science is by nature a reactionary science rather than proactive, in that almost all of the major research developments have occurred in other areas of scientific research and have then been applied to a forensic problem. This is primarily due to the lack of a strong research base within the forensic science community, which in turn is a reflection of the lack of funding.

1.5.1 History and current state of wildlife DNA forensic science

Non-human forensic investigation has suffered from a slow start due to lack of interest, the high cost of the development and validation of new forensic tests required by the courts, lack of funding, lack of expertise, and the low priority many police services will put to wildlife and environmental crime when compared to crimes against people or property.

Most tests relating to wildlife crime and non-human DNA were developed *ad hoc* as evidence and cases presented themselves. The development of DNA methods in wildlife testing is similar to other areas of scientific research and applies methods used in human identification, taxonomy and phylogenetics for purposes related to wildlife crime. The main problem when adapting tests that were designed originally for a different purpose is that they did not always work well with casework samples. Phylogenetic and conservation work generally had access to large amounts of DNA for their tests, with many using between 50 and 100 ng (1 ng $= 1 \times 10^{-9}$ g) of starting DNA, which was high quality and single source. This compares with casework samples that can be degraded, fragmented, composed of mixtures of many species and at low levels. For example, one species identification test for forensic use has been developed that is sensitive to the femtogram level (10^{-15} g) as this is more typical of the type of samples submitted for forensic examination (Tobe and Linacre, 2008). Further, research tests that give the correct result in 99% of the time might seem acceptable as a research tool, but with 1 case in 100 resulting in a potential miscarriage of justice.

One of the main set-backs in early non-human and wildlife forensic science was that untrained and/or unqualified scientists undertook casework, a phenomenon which can still take place today, but is relatively rare. It should be noted that wildlife forensic science was not alone in this, as other areas of non-mainstream opinion based sciences had the same problem. This situation can arise in different forms: police and solicitors can approach scientists with no forensic experience or training, but with experience in a loosely related subject when their other avenues of investigation fail; others market and put themselves forward as forensic experts with the assumption that forensic science is simple and straightforward; lastly, forensic scientists specialising in human DNA can assume that based on their human DNA training that they can undertake non-human DNA analysis without further training. All of these scenarios can result in poorly executed analyses and research, and this can be observed in some of the early published research and case reports (this will be discussed in greater detail later in the book). This poor overall standard of many areas of forensic science led to criticism of those areas, such as wildlife forensic science, published in 2009 by the US National Academy of Sciences report on forensic science (see Section 1.7).

Currently, wildlife forensic science is in a better position. The field, although still in its infancy, has been established for sufficient time that there are a growing number of trained experts who can properly carry out research and casework. This coupled with the rapid advancements in genetic sequencing and databases means that non-human forensic genetics is catching up to human forensic genetics. It also means that poor quality analyses and research can be spotted and confronted prior to publication. This is resulting in the development of high quality tests that are being readily accepted by the legal system.

1.5.2 Wildlife forensic science testing

DNA testing using restriction enzymes (see Chapter 3) to look at length differences between individuals led ultimately to the first methods of DNA fingerprinting in humans (Jeffreys *et al.*, 1985a, b). Prof. Sir Alec Jeffreys was studying a gene in grey seals when it was realised that methods used for looking at differences in seals could be applied to humans and also used to link family groups. It was not long before this same process was applied to other non-human samples, such as avian species (Burke and Bruford, 1987). The original method of human identification, termed DNA fingerprinting, used a process called multi-locus probes (see Chapter 3). This process used large amounts of DNA, took many days to complete and inter-laboratory comparison was not possible, hence there was a development firstly to a process called single locus probes and then to microsatellites (see Chapter 3). The forensic communities in many countries embraced DNA fingerprinting. There was a serious challenge in the United States (Lander, 1989) regarding the admissibility of DNA evidence in the case of People v Castro (Patton, 1990). The introduction of new methodologies into the US criminal courts required that the science met the Frye standard. The Frye test dates from 1923 (Frye v United States) when there was a challenge to the use of the polygraph (commonly called the lie detector). The conclusion was that novel technology had to meet general acceptance by the scientific community prior to acceptance by the court. Whether the first use of DNA fingerprinting in the United Kingdom met general acceptance is open to debate, but having been used in human identification there was a precedent for its use in non-human DNA typing. Wildlife investigation could piggy-back on the development of these and other methods used in both human identification and in taxonomic or evolutionary studies. The specific use of mitochondrial DNA for human identification (Wilson *et al.*, 1993), ancient DNA (Pääbo *et al.*, 1988) and evolutionary studies (Barton and Jones, 1983) left open the use of this type of DNA in species identification (Parson *et al.*, 2000).

Table 1.2 Relevant international societies and their conferences.

Societies	Conferences
International Society for Animal Genetics (ISAG) www.isag.us	ISAG meeting every two years: 33rd Cairns (2012) and 34th Xi'an (2014)
International Society for Forensic Genetics (ISFG) www.isfg.org	ISFG congress every two years: 25th congress in Melbourne (2013) and 26th congress in Krakow (2015)
Society for Wildlife Forensic Science www.wildlifeforensicscience.org	Meetings every three years: 1st conference, Jackson Hole (2012)

Forensic wildlife testing relies on financial assistance from organisations that fund research, although the actual funding is all too frequently the remit of 'another' funding body[1]. Despite the lack of funding there has been a growing number of research publications and case reports in the scientific literature. There are a number of peer-reviewed international journals that accept wildlife forensic science research and case reports. These include for example *Forensic Science International* and *Forensic Science International Genetics*, the journal of the International Society for Forensic Genetics (ISFG, www.isfg.org), which recently appointed an associate editor specifically to handle papers in this field; *Journal of Forensic Sciences*; *Science and Justice*; and *Forensic Science, Medicine and Pathology* also accept non-human articles. Associated with these publications there are a number of international conferences hosted by professional societies such as the triennial meetings of the Society for Wildlife Forensic Science (SWFS, www.wildlifeforensicscience.org), ISFG and the International Society for Animal Genetics (ISAG, www.isag.us). A number of relevant societies and their related symposia are provided in Table 1.2.

The development of DNA testing in human identification led to much research and investment by commercial suppliers to meet this need. Commercial companies such as Applied Biosystems (now Life Technologies) and the Promega Corporation developed DNA-based 'kits' for the purpose of human identification and performed their own validation studies. The laboratories using these kits need only perform simple verification tests to ensure that the results met the expected outcomes. Little such commercial investment was forthcoming in non-human DNA testing, although tests for cattle, dogs and horses (FinnZymes Diagnostics and ABI Stockmarks® for instance) were produced. Wildlife DNA typing, be it for species identification or linking a sample to an organism, all too often has borrowed methods from other fields of biology.

[1] It should be noted that the Leverhulme Trust (UK) funded research assisting the authors.